ADVENTURES IN RADIOISOTOPE RESEARCH

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The Collected Papers of
GEORGE HEVESY
in Two Volumes

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CONTENTS

INORGANIC AND PHYSICAL CHEMISTRY

Volume One

Analytical Applications

1 The Solubility of Lead Sulphide and Lead Chromate (with F. Paneth) 31

2 Platinum Black (with M. Somya) 36

3 Lead Content of Rocks (with R. Hobbi) 43

Activation Analysis

4 The Action of Neutrons on the Rare Earth Elements (with H. Levi) 47

5 Artificial Activity of Hafnium and Some Other Elements (with H. Levi) 63

Electrochemistry

6 The Problem of the Isotopic Elements (with F. Paneth) 75

Interchange Studies

7 The Velocity of Dissolution of Molecular Layers (with E. Rona) 89

8 The Exchange of Atoms Between Solid and Liquid Phases 97

9 Intermolecular Exchange of Atoms of the Same Kind (with L. Zechmeister 103

Selfdiffusion

10 Self-diffusion in Solid Lead (with J. Groh) 110

11 Self-diffusion in Solid Metals (with A. Obrutsheva) 114

12 The Heat of Relaxation of the Lead Lattice (with W. Seith and L. Keil) 116

13 Diffusion in Metals (with W. Seith) 122

14 Application of Radioactive Recoil in Diffusion Measurements (with W. Seith) 127

Tracers In The Search For Unknown Stable Elements

15 Search for an Inactive Isotope of the Element 84 (Polonium) (with A. Günther) 140

LIFE SCIENCE

Application of Radioactive Tracers Occurring in Nature

16 Radiochemical Method of Studying the Circulation of Bismuth in the Body (with J. A. Christiansen and S. Lomholt) 143

17 Radiochemical Method of Studying the Circulation of Lead in the Body (with J. A. Christiansen and S. Lomholt) 145

Skeleton Studies

18 Radioactive Indicators in the Study of Phosphorus Metabolism in Rats (with O. Chiewitz) 149

19 Studies on the Metabolism of Phosphorus in Animals (with O. Chiewitz) 152

20 Investigations on the Exchange of Phosphorus in Teeth Using Radioactive Phosphorus as Indicator (with J. J. Holst and A. Krogh) 168

21 Rate of Rejuvenation of the Skeleton (with H. Levi and O. Rebbe) 191

22 Retention of Atom of Maternal Origin in the Adult White Rat 198

23 Rate of Renewal of the Fish Skeleton 204

24 Conservation of Skeletal Calcium Atoms Through Life 217

25 Path of Atoms Through Generations 234

26 Note on the Chloride Content of the Mineral Constituents of the Skeleton 241

Phosphatides

27 The Formation of Phosphatides in the Brain Tissue of Adult Animals (with L. Hahn) 246

28 Lecithenaemia Following the Administration of Fat (with E. Lundsgaard) 255

29 Formation of Phosphatides in Liver Perfusion Experiments (with L. Hahn) 258

30 Rate of Penetration of Phosphatides Through the Capillary Wall (with L. Hahn) 262

31 Origin of Phosphorus Compounds in Hen's Eggs (with L. Hahn) 273

32 The Origin of the Phosphorus Compounds in the Embryo of the Chicken (with H. Levi and O. Rebbe) 293

33 Formation of Milk (with A. H. W. Aten) 304

34 Formation of Lecithin, Cephalin and Sphingomyelin (with L. Hahn) 309

35 Turnover of Phosphatides (with G. Eliott) 346

Acid Soluble Phosphorus Compounds

36 Molecular Rejuvenation of Muscle Tissue (with O. Rebbe) 366

37 Rate of Renewal of the Acid Soluble Phosphorus Compounds of the Rabbit (with L. Hahn) 369

38 Circulation of Phosphorus in the Frog (with L. Hahn and O. Rebbe) 384

Fatty Acids

39 Turnover Rate of the Fatty Acids of the Liver (with R. Ruyssen and L. M. Beeckmans) 403

40 Effects of Dinitro-Cyclo-Pentylphenol on the Incorporation of Labelled Acetate Carbon (^{14}C) Into Tissue Fractions (with L. M. Beeckmans and H. Casier) 408

41 Determination of the Rate of Renewal From the Rate of Disappearance of Labelled Molecules 417

Permeability Studies

42 Rate of Penetration of Ions Through the Capillary Wall (with L. Hahn) 425

43 Rate of Passage of Water Through Capillary and Cell Walls (with C. F. Jacobsen) 437

44 Rate of Penetration of Phosphate into Muscle Cells (with O. Rebbe) 443

45 The Effect of Excitation on Nerve Permeability (with H. Euler and U. Euler) 454

46 Note on the Inorganic Phosphate of Blood Plasma (with G. Elliot and L. Hahn) 460

47 Fate of the Sulphate Radical in the Animal Body (with A. H. W. Aten) 465

48 Diplogen and Fish (with E. Hofer) 468

49 Interaction of Plasma Phosphate with the Phosphorus Compounds Present In The Corpuscles (with A. H. W. Aten) 471

50 Rate of Penetration of Ions Into Erythrocytes (with L. Hahn) 493

Volume Two

Labelling of Red Corpuscles

51 A Method of Blood Volume Determination (with L. Hahn) 517

52 Determination of the Red Corpuscle Content (with K. Zerahn) 523

53 Thorium B Labelled Red Corpuscles 531

Clinical Investigations

54 Elimination of Water from the Human Body (with E. Hofer) 536

55 Excretion of Phosphorus (with L. Hahn and O. Rebbe) 540

56 Potassium Interchange in the Human Body 553

57 The Red Corpuscle Content of the Circulating Blood Determined by Labelling the Erythrocytes with Radio-Phosphorus (with K. H. Köster, G. Sørensen, E. Warburg and K. Zerahn) 561

58 Application of ^{42}K Labelled Red Corpuscles in Blood Volume Measurements (with G. Nylin) 573

59 Application of "Thorium B" Labelled Red Corpuscles in Blood Volume Studies (with G. Nylin) 580

60 Cancer Anaemia 597

Iron Metabolism

61 Effect of Adrenaline on the Interaction Between Plasma and Tissue Constituents (with G. Dal Santo) 610

62 Effect of Irradiation on Hemin Formation (with R. Bonnichsen) 624

63 Haemoglobin Present in the Nuclear Fraction of the Liver (with R. Bonnichsen, G. Ehrenstein and J. Schliack) 634

64 Application of Isotopic Indicators in Haematology 639

65 Note on the Determination of Radioiron (with K. Agner and R. Bonnichsen) 651

66 Embryonal Iron Turnover (with G. v. Ehrenstein) 655

Nucleic Acids

67 Rate of Formation of Nucleic Acid in the Organs of the Rat (with J. Ottesen) 663

68 Rate of Renewal of Ribo- and Desoxyribo Nucleic Acids (with E. Hammarsten) 673

69 Turnover of Ribosenucleic Acid in the Jensen-Sarcoma of the Rat (with H. Euler and W. Solodkowska) 680

70 Life-Cycle of the Red Corpuscles of the Hen (with J. Ottesen) 688

Studies in Radiation Biology

71 Effect of X-rays on Nucleic Acid Formation in the Jensen- Sarcoma (with H. Euler) 692

72 The Effect of X-rays on Nucleic Acid Formation in the Organs Of The Rat (with L. Ahlström and H. Euler) 721

73 Turnover of Nucleic Acid in Retrogade Sarcomata (with L. Ahlström and H. Euler) 731

74 The Indirect Effect of X-rays on the Jensen-Sarcoma (with L. Ahlström and H. Euler) 744

75 Attempts to Find Products Blocking Nucleic Acid Formation in the Circulation of an Irradiated Organism (with L. Ahlström, H. Euler and K. Zerahn) 758

76 Fate of The Nucleic Acid Introduced into the Circulation (with L. Ahlström and H. Euler) 761

77 Formation of Nucleic Acid in Sarcoma Slices. (with L. Ahlström and H. Euler) 770

78 Application of Labelled Substrates in the Study of Enzymic Processes (with L. Ahlström and H. Euler) 783

79 Effect of X-Rays on the Incorporation of Carbon-14 into Desoxyribonucleic Acid 791

80 Effect of X-Rays on the Incorporation of Carbon-14 into Animal Tissue 793

81 Effect of X-Rays on the Incorporation of 14-C into Tissue Fractions of the Mouse (with G. Dreyfus) 795

82 Effect of Muscular Exercise and of Urethane Administration on the Incorporation of Carbon-14 into Animal Tissue 821

83 Effect of Irradiation by X-Rays on the Exhalation of Carbon Dioxide by the Mouse (with A. Forssberg) 825

84 Effect of X-Rays and Hormones on Resorption Rate of Injected $Na^{14}HCO_3$, (with A. Forssberg) 828

85 Note on the Effect of X-Rays and Hormones on the Resorption Rate of Injected $Na^{14}HCO_3$ (with A. Forssberg) 838

86 Effect of Irradiation with X-Rays on the Catabolism of Methylalcohol in the Mouse 841

87 Effect of Irradiation with X-Rays on the Catabolism of Ethylalcohol in the Mouse 847

88 Radioactive Tracers in Radiobiological Studies. The Thirty-Sixth Silvanus Thompson Memorial Lecture 851

Botanical Studies

89 The Absorption and Translocation of Lead by Plants 876

90 Atomic Dynamics of Plant Growth (with K. Linderstrøm-Lang and C. Olsen) 884

91 Exchange of Phosphorus Atoms in Plants and Seeds (with K. Linderstrøm-Lang and C. Olsen) 887

92 Interaction Between the Phosphorus Atoms of the Wheat Seedling and the Nutrient Solution 891

93 Exchange of Nitrogen Atoms in the Leaves of the Sunflower (with K. Linderstrøm-Lang, A. S. Keston and C. Olsen) 905

94 Zinc Uptake by Neurospora (with I. Andersson-Kottö) 910

95 Phosphorus Exchange in Yeast (with K. Linderstrøm-Lang and N. Nielsen) 916

96 Potassium Interchange in Yeast Cells (with N. Nielsen) 918

97 Note on the Number of Pollen Grains Identified in the Fruit of the Aspen (with C. Eklundh-Ehrenberg and H. Euler) 924

Lectures

98 Some Applications of Isotopic Indicators. Nobel Lecture 928

99 The Application of Radioactive Indicators in Biochemistry. Faraday Lecture 961

100 Historical Progress of the Isotopic Methodology and its Influences on the Biological Sciences. Read at the Turin Meeting of the Society of Nuclear Medicine 997

Index 1039

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A SCIENTIFIC CAREER

George Hevesy,
Ph. D. (hon.), Ph. nat. D. (hon.), D. Sc. (hon.), Sc. D. (hon.) M. D. (hon.)
Jur. D. (hon.).

I was born in Budapest the 1st of August, 1885. After terminating my studies at the Gymnasium of the Piarist Order in that city, I studied a short time in Budapest and Berlin and later in Freiburg, mainly chemistry and physics, where I took my degree in 1908 an. The subject of my doctoral thesis was the interaction between metallic sodium and molten sodium hydroxide, an interaction responsible for a poor yield often obtained when producing sodium by electrolysis of molten sodium hydroxide.

Being interested in high-temperature chemistry, I proceeded to Zürich after obtaining my degree to work under Richard Lorenz, at that time the most eminent representative of that branch of science. The Technische Hochschule of Zürich was in those days, as it is today, a great place of learning and teaching. The Swiss chemical and pharmaceutical industry could not have reached its present high standard it represents today without the aid of a great number of able chemists, most of them trained at the Technische Hochschule of Zürich. When I joined this institution, the permanent head of the chemistry department was Willstätter.

Einstein's First Lecture

Shortly after my arrival at Zürich, Einstein was appointed associate professor of theoretical physics on the University. I was one of the audience of about twenty who attended his inaugural lecture on the determination of the ratio of charge and mass of the electron. (Einstein left after a few years for Prague and returned later to Zürich to fill the chair of theoretical physics on the Technische Hochschule.) When he visited our laboratory, I had the privilege to show him around. I remember vividly his astonishment when shown a hydrogen electrode. He thought such an electrode to be only a theoretical concept.

Twenty-three years later, after terminating my Baker lectureship on the Cornell University at Ithaca, I met Einstein in Pasadena. I visited a

barber shop whose owner, a son of the City of Constance, praised the beauties of life in California, mentioning that his only wish in life was to be permitted once to cut Einstein's hair. I told him that this wish would not be easy to fulfill as, according to rumors, Mrs. Einstein used to perform this work. When I told Einstein about the barber's wish, he remarked : "Da er sich auf Ihrem Kopfe nicht austoben konnte, wollte er meinen Kopf haben" ("As he could not sufficiently exercise himself on your head [I had poor hair] he wants to have mine").

Einstein talked repeatedly to me on the problem of causality. He disagreed with Bohr's views on this topic and asked me to convey his objections to Bohr. He wished an explanation on a classical basis.

When Lorenz left Zürich for the University of Frankfurt (I was later, after his death, asked to fill his chair). Willstätter called on me to make the short statement : "In Germany the assistant belongs to the professor, in Switzerland to the laboratory—you stay here." I did not, as I got much interested in the catalytic synthesis of ammonia by Haber, a discovery which at that date rightly impressed all those interested in chemistry.

My monthly salary in Zürich corresponding to $36 was entirely adequate, as I was charged $15 a month for a very nice room and two good meals a day. When I was promoted to a "first assistant", I was told that my salary would be raised to $60 a month, the highest pay ever allotted to an assistant.

When I was leaving the laboratory one evening together with Willstätter, he told me that he was moving to Berlin to take over one of the Kaiser Wilhelm Institutes. I asked him, much astonished, why he was leaving. He was the permanent head of the chemistry faculty and had a very fine laboratory, and postgraduate students from all over the world were anxious to work under his guidance. His answer was : "If the fatherland calls, it is my duty to go." Thirty-two years later I was present at the meeting of the Danish Academy of Sciences when the president, S. P. L. Sörensen, death written on his face, read a letter from Willstätter requesting that the *Proceedings* of the Academy should no more be sent to him. Willstätter went on, saying : "I have no home any more. I have lost all my belongings, which I do not mind much. What chagrins me is that I lost my fatherland."

Haber wished me to work in another field than that of catalytic synthesis. I was to investigate whether or not oxidation of molten zinc is accompanied by emission of electrons. No one in Haber's institute had experience in the field of the conductivity of electricity in gases. I proposed therefore to Haber that I proceed to England to acquire some knowledge in this new field of physics and return later to his laboratory. Haber entirely shared my view, and I left in the first days of January, 1911, for Manchester to work under Rutherford.

Years with Rutherford

The physics laboratory of the University of Manchester was housed in a spacious building. The chief equipment of the institute was electroscopes built with cocoa cans, sealing wax, sulphur rods, gold leaves, and reading microscopes. Once adjusted, the electroscopes were not permitted to be cleaned, and the smokey atmosphere of Manchester left its visible marks all over the laboratory. The years I had the privilege to spend in Rutherford's laboratory in Manchester, between 1911 and 1914, witnessed some of the greatest discoveries in the history of physics. I could follow from close quarters the discovery of the atomic nucleus and how Rutherford devised, carried out, and interpreted the results of experiments. All this was done with the greatest ease, without visible effort.

Niels Bohr came to Manchester in 1912. He recently remarked in an after-dinner speech that I was the first-one he met when he entered Rutherford's institute. Rutherford—and not he alone—soon realized Bohr's genius. When I was enjoying Rutherford's hospitality one Sunday afternoon, soon after the discovery of the atomic nucleus, I happened to ask him about the origin of β-rays. The α-rays clearly originated from the nucleus, but what about the origin of β-rays? Rutherford answered promptly, "Ask Bohr", and the answer was at once given by the latter, emphasizing the difference between nuclear and non-nuclear β-particles. Bohr was however not always easy to understand. When he briefly stated, "Argon is not the right argon", he made a statement that was at that date not easy to interpret. It was then, in 1912, already clear to him that it is not the mass number but the atomic number that is decisive for the place of an element in the periodic system. Soon after, this fact was decisively brought out by Moseley's work. I consider myself lucky to have had the opportunity to help Moseley set up the first X-ray spectrograph. We turned to the steward of the chemistry department, Mr. Edwards, who handed us a beautiful, very large potassium ferricyanide crystal which found application in Moseley's spectrograph. A magnetic device served to bring small metal disks covered with the element to be investigated into the electron beam, which had to excite the X-rays. Moseley's fundamental work brought out, among other things, that, while the atomic weight of argon is higher than that of potassium, its atomic number is not—that "argon is not the right argon", as stated by Bohr previously. Moseley has also shown that the anomaly of the positions of tellurium and iodine in the periodic system disappears if we consider the atomic number instead of the atomic weight.

When I arrived at Manchester, Rutherford wished me to study the solubility of actinium emanation in various liquids. It was not an easy task in view of the short life of this emanation, now called actinon, the half-life of which is four seconds only. This was, however, a very good

school to learn the handling of short-lived substances. I later became engaged with the study of the electrochemical properties of radioelements of unknown chemical character and the measurement of their valency from diffusion data.

The early origin of the famous Geiger-counter goes back to those Manchester days as well. Rutherford and Geiger counted α-particles by making use of a galvanometer which registered the arrival of each α-particle. The ionization produced was magnified by using the principle of production of ions by collision. The much more difficult task of counting β-particles was solved later, after the first World War, by Geiger, then at Kiel.

When I was in Manchester, Rutherford was much interested to come into the possession of a strong radium D sample. Large amounts of radium D were stored in the laboratory, but imbedded in huge amounts of lead. The great German chemist Haber intended to pay Germany's war debts after the first World War by extracting gold from the ocean. First he undertook to check the correctness of the available gold analyses of sea water. He found the gold content of the ocean to be very much lower than previously found. He summarized the depressing results of his expedition by stating : "Dilution is the death of all value". Rutherford could have made the same remark when glancing at the hundreds of kilograms of lead chloride extracted from pitchblende and presented to him by the owner of the Joachimsthal mines, the Austrian government.

Radioactive Tracers

One day I met Rutherford in the basement of the laboratory where the lead chloride was stored. He addressed me by saying : "If you are worth your salt, you separate radium D from all that nuisance of lead." Being a young man, I was an optimist and felt sure that I should succeed in my task. Trying during a year all sorts of separation methods and making the greatest efforts, it looked sometimes as if I succeeded, but I soon found out that it was radium E, the disintegration product of radium D, a bismuth isotope, which I separated. The result of my efforts was entire failure. To make the best of this depressing situation, I thought to avail myself of the fact that radium D is inseparable from lead, and to label small amounts of lead by addition of radium D of known activity obtained from tubes in which radium emanation decayed. From such tubes pure radium D can be obtained.

It was the Vienna Institute for Radium Research which owned in those days by far the greatest amount of radium and, correspondingly, of radium emanation. This fact induced me to interrupt my stay in Manchester and to proceed to Vienna. In the Vienna Institute there were

very large amounts of lead chloride, obtained from pitchblende as well, and Paneth, assistant at the Institute, unaware of my efforts at Manchester, made very extensive studies to achieve separation. His great efforts were as abortive as mine. At my suggestion we associated in the application of labelled lead.The first use of this method, early in 1913, was the determination of the solubility in water of sparingly soluble salts such as lead sulphide and lead chromate. We then proceeded to study the electrochemistry of bismuth and lead by making use of the method of radioactive indicators. We could show, among other things, that Nernst's law of the dependence of the electrode potential on the ionic concentration is valid even at exceedingly low concentrations. Paneth then directed his interest toward the interaction of the lead ions present in the surface layer of lead sulphate and the labelled lead ions of the surrounding solution. I studied the interaction of the lead atoms of a lead foil and also of lead peroxide with the lead ions of a solution, employing labelled lead foils and non-radioactive lead salt solution, or vice versa. In the last of the numerous joint investigations with Paneth, we succeeded in preparing visible amounts of radium D from radium emanation. By comparing the electrode potential of radium D peroxide with that of lead peroxide, we were able to show that these cannot be distinguished from each other.

During my stay in Vienna, I undertook balloon ascents in the company of Hess and Paneth. On one of his trips Hess took an electrometer with him to follow the change in the ionization of the air with height. He assumed this ionization to be due to terrestrial radiation and correspondingly expected it to decrease with height. The opposite, however, was found to be the case. With such simple means and without much effort this observation led to the discovery of cosmic radiation.

Madame Marie Curie

When passing through Paris on the way to Manchester, I never failed to call on Marie Curie and I was always sure to find her amidst experimental work. She was usually surrounded by several girl assistants precipitating or crystallizing preparations. The only protection that she used was finger caps of rubber. When engaged with the concentration of actinium from rare-earth samples, she generously presented me with an actinium preparation. I consider this specimen one of my most precious belongings. As the years pass by, the bottle containing the radioactive sample is getting more and more coloured, indicating the many years which have elapsed since I met this most remarkable personality and great pioneer.

At a later visit to the Institut de Radium, I met Joliot, who was then a young assistant engaged in the study of the electrochemistry of polo-

nium, which many years earlier was in the center of interest of Paneth and myself. Also, Irène Curie worked in the laboratory of her mother. When I saw her in 1938, she mentioned that by neutron bombardment of thorium she had obtained a lanthanum-like radioactive body. I asked her if she was sure that this substance was not actinium. She answered that she was pretty sure she was dealing with an element much lighter than one of the radioactive disintegration series.

A few months later Otto Hahn and Strassman made their fundamental discovery of nuclear fission. I first met Hahn in Vienna in 1913. Already at that date he had made such important discoveries as the existence of radiothorium and mesothorium and the separation of radioelements by making use of the recoil phenomenon. The years to come, brought new discoveries of great importance, many of them in collaboration with Lise Meitner. When I asked Rutherford in 1912 whom of his students he considered to be the most merited one, he answered without hesitation "Otto Hahn".

On my way to Manchester I usually stopped in London. On such an occasion I had the opportunity of being present in the House of Commons at the introduction of the much discussed budget by Lloyd George, then Chancellor of the Exchequer, who characterized his introduction of heavy death duties and other taxes as "bringing rare and refreshing fruit"!

I was also present when J. J. Thomson in April, 1913, delivered his Bakerian Lecture in the Royal Society on the two neon parabolas obtained in his positive ray studies. He did not make any allusion to the analogy between the two neons and the isotopes in the field of radioactivity. This omission induced me to write to him drawing his attention to the analogy between the two kinds of neon, on one hand, and radium D and lead, on the other. He stated in his answer that he did not share my view. While not adopting the view that the heavier constituent of neon was a compound NeH_2, which could have given the observed atomic weight within the limits of experimental error, Thomson was not convinced that this explanation was absolutely excluded. As Lord Rayleigh remarks in *The Life of Sir J. J. Thomson*, he had always been haunted by this suspicion about hydrogen compounds and, for that reason, hesitated for a time to accept Aston's later results about isotopes of other elements. When we were on a ski-trip at Finse in Norway, Aston related that when he first succeeded in getting two lines on a mass spectrum photograph — one indicating ^{35}Cl, the other ^{37}Cl — Thomson refused to look at the photograph, which, Aston added, was the most beautiful one he ever obtained. Aston was an ingenious and most merited experimenter, who was the first one to prove the complexity of the common elements.

In 1914 Moseley moved to Oxford and, being much interested in X-ray spectroscopy, I intended to work with him. We wanted to study

the X-ray spectrum of the elements 68 through 72. I was already in Holland on the way to Oxford when the first World War broke out, soon followed by the tragic death of Moseley. While talking on a field telephone at Gallipoli, a bullet struck the head of this ingenious and most remarkable man. By a curious coincidence, I was to occupy myself extensively with the X-ray spectrum of the element 72 eight years later.

Measurement of Self-diffusion

As I was at that time a Hungarian subject (I am now a Swedish citizen) I was drafted into the Austro—Hungarian army. I spent much of that time as technical supervisor of electrolytic copper works. While located in Carpathian plants, I fitted up a laboratory on a very modest scale and studied the difference in the chemical behaviour of the active deposit of thorium when present in ionic and colloidal state. For several months after the end of the war it was not possible to leave Hungary. During these months I started with my friend Groh to study self-diffusion in molten and in solid lead, using radium D as an indicator. We fused a radiolead-rod on to the top of an inactive lead-rod, heated this solid system to 200°—300°, and determined the dislocation of the radium D atoms. From the extent of dislocation, the rate of self-diffusion of lead was calculated. This early, rough method was improved later during my stay in Copenhagen. Together with the Russian scientist Mrs. Obrutsheva, we condensed the lead isotope thorium B on top of a lead foil and counted the number of scintillations produced by the α-rays emitted by the disintegration products of thorium B. Upon heating of the sample, thorium B diffused into the lead foil, resulting in a reduction of the number of scintillations observed. From this reduction, the diffusion rate of lead in lead could be calculated. Heisenberg, then lecturer in Copenhagen, very kindly at that time helped us with these calculations. Later on in Freiburg, Seith and myself made use of the recoil phenomenon to measure self-diffusion in lead. This is an exceedingly sensitive method, which permitted measurement of diffusion rates as slow as 10^{-14} cm^2/day.

The Rockefeller Foundation started to support my investigations in 1930 and continued most generously to do so for the following twenty-five years.

Niels Bohr's Institute

In the first days of May, 1919, I left for Copenhagen to spend some time with Niels Bohr at the charming summer house in Tibirke. At that time his premises were at the Technological Institute of Copenhagen, from which he directed the construction of his new institute. When he

had to decide on a name for the new institute, he hesitated between "Theoretical Physics" and "Atomic Physics". His choice fell on the first one ; he felt that the latter might be too exacting and possibly too special as well. In front of the Technological Institute there is a statue of Olaus Römer, the first physicist to measure the velocity of light. I once pointed out when passing this monument that space is available for a future monument of Niels Bohr. My companion smiled at this remark. Today he would not smile any more.

It was settled with Bohr that I should be back in Copenhagen in the spring 1920, to start activities at the new institute which was to be opened by that date. I spent the remaining six months with my friend Zechmeister in Budapest carrying out exchange studies by the application of radioactive indicators. When dissolving in water both 1 mol of labelled lead nitrate and 1 mol of non-radioactive lead chloride, or labelled lead chloride and non-radioactive lead nitrate, after separation of the two compounds, we found the radioactivity equally distributed between $PbCl_2$ and $PbNO_3$. When dissolving non-radioactive tetraphenyl lead and radioactive lead nitrate, after separation all radioactivity was conserved in the nitrate sample, as the lead atoms of tetraphenyl lead are not exchangeable. When I met Svante Arrhenius in 1922 he told me about his interest in the above-mentioned work. The experiments with labelled lead chloride and non-labelled lead nitrate, or vice versa, are the most direct proof of the correctness of the theory of electrolytic dissociation.

After the war, I was anxious to go to England as soon as possible. The atmosphere at that time, however, radically differed from the one that prevailed after the second World War. When in 1921 I wrote from Copenhagen to Rutherford, a very liberal man, that I wished to visit England, he answered that it was still too early for a former enemy to come to England. In 1923, however, when he was elected president of the British Association meeting which was to take place in Liverpool, he invited me to address that meeting on the discovery of hafnium. I recall a lunch party at Liverpool in which Lord and Lady Rutherford, Niels Bohr, Millikan, Aston, Coster, and myself took part. Lady Rutherford remarked that this party included four Nobel Laureates. Rutherford added, "And some embryos".

Rudolf Schoenheimer

During my stay in Liverpool I was told about the work of Blair-Bell who claimed a successful cancer therapy through administration of lead compounds. These results induced me, when I worked at the University of Freiburg some time later, to study the distribution of labelled lead compounds between cancerous and normal tissue. A study

of the distribution of labelled lead and bismuth in healthy rabbits was carried out earlier, in 1924, in Copenhagen. I approached the great pathologist Aschoff to delegate one of his collaborators to help us in our work. He first delegated the director of a hospital on the island of Formosa and later, to help him, his chief chemist, Rudolf Schoenheimer. This was Schoenheimer's first experience with tracer work, a field to which he later, jointly with his eminent colleague Rittenberg, made unsurpassed contributions. Schoenheimer was already at that date a very nervous man. He moved his limbs incessantly, smoked cigarettes, and consumed coffee on a much too liberal scale. When our work was finished, he left Freiburg and I never saw this most merited man again.

Separation of Isotopes

When I went to Copenhagen in the spring of 1920, Bohr's institute was not yet ready. I associated with the eminent physicochemist Brønsted to investigate a problem of great interest to both of us, namely, the partial separation of isotopes on a preparative scale. We based our procedure on the more rapid rate of evaporation of the lighter isotope from a liquid. We distilled mercury in high vacuum at 40° and prevented the more rapidly evaporating lighter isotopes from being reflected back into the liquid mercury by freezing them on a glass surface cooled with liquid air. By repeating this process some hundred times, we obtained a light and a heavy mercury fraction. The results were controlled by both density measurements and atomic weight determinations, the latter being carried out by Hönigschmid in Munich.

When partially separating the isotopes of chlorine, we made use of the above-mentioned method again. We distilled concentrated solutions of hydrochloric acid in water and obtained several liters of water containing hydrochloric acid with different isotopic chloride composition. I suggested to Brønsted that he have a look at the density of the water obtained. He objected to my suggestion, as shortly before two distinguished German chemists, Vollmer and Stern, had searched without success for other isotopes of hydrogen and oxygen than ^{1}H and ^{16}O. These workers carried out diffusion experiments through porous membranes. When I, after Urey's discovery of deuterium, reminded Brønsted of my suggestion, he answered: "A discovery like this should not be made fortuitously; it should be based on careful considerations like Urey's."

Bohr was highly interested in our separation experiments and keenly followed our progress. Bohr's greatness is due not only to his ingenuity but to the unique catholicity of his interests, his sagacity, and his immense conscientiousness. When as a young man he intended to publish his first "letter" to the editor of *Nature*, he wrote the note over and over again. Finally his brother, who later achieved fame as a great mathema-

tician, suggested he should now mail the "letter". Niels Bohr was shocked by this suggestion, since, he said, this was the first trial of the first concept of the "letter." In this spirit all his papers were written.

How fabulously far-sighted Bohr was, is seen from a letter which the present writer addressed to Rutherford after the Birmingham meeting of the British Association for Advancement of Science from Budapest the 14th October 1914.

"The meetings on Monday and Tuesday have been very interesting. It is a most remarkable fact that Aston succeeded to separate the two Neons by diffusion and gave a definite proof that elements of different atomic weights can have the same chemical properties. Thomson came in his paper on X_3 to the conclusion that the latter is a polymerized hydrogen, a kind of H_3 (like O_3). In the following discussion Bohr—in his usual modest way — suggested the possibility that X_3 being an H atom with one central charge, but having a three-times heavier nucleus than hydrogen. He suggested to let a mixture of H and X_3 diffuse through palladium and try if it is possible to separate them, as the heavier X_3 atom has to diffuse much slower.

"Bohr had not been understood properly and Thomson gave a rather quick answer, saying — after a brief consultation with Ramsey — that Bohr's suggestion is useless, for not molecules, but the atoms of H diffuse through Palladium. Certainly, but this was just Bohr's point.

"The general appearance was, that he told something highly ingenious and Bohr something very stupid. Just the contrary was the case. So I felt bound to stick up for Bohr and explained the meaning of Bohr's, suggestion in more concrete terms, saying that Bohr's suggestion is that X_3 is possibly a chemically non-separable element from Hydrogen . . . Of course not very probable, but still a very interesting suggestion; which should not be quickly dismissed" . . . 26 years later Tritium was discovered.

Simultaneously with the isotope separation studies, I carried out among other things some tracer-work on the interchange between the atoms of lead compounds and lead, all in molten state.

In 1921 Bohr's institute was opened. Those working at the institute at its start were, besides its director, H. Kramers, H. M. Hansen, I. C. Jacobsen, James Franck, who was invited for a short visit, and myself. In my first study at the institute I measured the conductivity first of a single crystal of sodium nitrate and then after it was molten and resolidified into a polycrystalline mass. This crystalline conglomerate was found to have a specific conductivity, fifty times higher than the single crystal. From this result it was concluded that deviations from the ideal crystalline state promote electrolytic conductivity. While increase of temperature produces a reversible loosening of the lattice, we are

here faced with an "irreversible loosening" of the crystal structure. This was a most modest beginning in a field that later proved to be of great importance.

Hafnium

Bohr's first fundamental papers, published in 1913, in which the quantum theory of the atomic structure was introduced, dealt only with the structure of the atoms of hydrogen, helium, and lithium. In January, 1922, I learned during a walk together with him that he now had extended his theory to the entire periodic system, giving among other things an explanation of the appearance of the rare-earth elements in that system. Their number according to his theory was restricted to fourteen, from which it followed that the unknown element 72 cannot be a rare earth, it has to be a homologue of the titanium group.

In the summer of that year I became interested in geochemical problems. Returning to Denmark, I proposed to Coster, who previously had studied X-ray spectroscopy with Siegbahn in Lund, that he should teach me the technique and that we ought at the same time to have a look at zirconium minerals for the missing element 72. The first spectrum obtained by him demonstrated the presence of the element in zirconium minerals, and further studies revealed its presence in all commercial zirconium samples, which indicated a very close kinship between zirconium and the new element hafnium. By a very protracted fractional crystallization of ammonium zirconium hexafluorides, hafnium could be prepared in a pure state.

The discovery of hafnium was not accepted without opposition. Urbain, in Paris, a few years earlier crystallizing crude ytterbium salts, observed twenty-six optical spectral lines not shown by the initial sample. He ascribed these lines to the presence of the previously unknown element 72 in his sample. After the discovery of hafnium, it was, however, demonstrated that none of these lines is to be found in the spectrum of hafnium. In spite of this fact, Urbain upheld his claim to have discovered element 72. Rutherford took great interest in our work—all our extensive correspondence with *Nature* passed through his hands—and suggested that I should send a paper on the chemistry of hafnium to the editor of *Chemical News*. He remarked in his letter that the editors of this periodical were strongly pro-French and I should not mind if they refused to publish my paper. In Sheffield, my friend the physicist Lawson ("interned" as a prisoner of war in the Institute of Radium Research of Vienna) handed my contribution over to the editor of *Chemical News*, Professor Wynne. He remarked that he was pleased with the paper but they might have something to say about the name "hafnium," adding: "We adhere to the original word Celtium given to

it by Urbain as a representative of the great French nation which was loyal to us throughout the war. We do not accept the name which was given it by the Danes who only pocketed the spoil after the war." The paper was, however, published by *Chemical News* without remark.

Another opposition to the discovery of hafnium came from London. Alexander Scott, the chief chemist of the British Museum, could not identify a fraction of a sample of an Australian titaniferous sand. After our discovery was announced, he thought this fraction to be hafnium. Scott's paper induced the *Times* to publish in its February 2, 1923, issue an editorial under the title "Hafnium", stating: "Science is, and doubtless should be, international, but it is gratifying that this chemical achievement, the most important since the late Sir William Ramsay isolated helium in 1895, should have been the work of a British chemist in a London laboratory." Scott's sample, sent us for investigation, did not contain a trace of hafnium or zirconium.

The determination of the hafnium content of a great number of zirconium minerals and historical zirconium samples was a fascinating task. Berzelius determined the atomic weight of zirconium by analyzing its sulphate. This method supplies too low values for the atomic weight. This error was, however, compensated by the presence of hafnium, almost twice as heavy as zirconium, in his sample. Venable in South Carolina, who spent many years with the determination of the atomic weight of zirconium, applied a modern method devised by Richards at Harvard. He could not find the reason why his determination led to a clearly too high value. After the discovery of hafnium, he sent us a sample of his zirconium, and, after taking into account its quite appreciable hafnium content—which we determined—he could correct the presence of hafnium in his sample and arrive at a precise value for the atomic weight of zirconium.

Through my work with hafnium I came into contact with the great Austrian chemist Auer von Welsbach. He invested a part of his very substantial royalties obtained for his patent of cerium-iron alloys (applied in cigar-lighters among other things) in a beautiful estate in Carinthia on which he built a castle. The rough crystallization of rare earths was carried out in one of his nearby situated works, and the final crystallization was done by himself in his castle. He was at that date and for many years to come the only man who possessed highly purified samples of all elements of the rare-earth group. When staying with him, he expressed his astonishment that when separating hafnium from zirconium I had chosen to handle large amounts of fluorides, which are highly unpleasant compounds to work with. He achieved all his great success in the field of rare-earth chemistry by crystallizing double- sulfates. We found out later that there is no significant difference between the solubility of zirconium and hafnium double- sulfates, and if we had chosen

to crystallize these compounds, we would not have been able to separate hafnium from zirconium. All hafnium commercially available for the next twenty-five years was prepared by crystallizing the double fluorides.

V. M. Goldschmidt

Auer von Welsbach presented me with small samples of octohydrosulphates of all elements of the rare-earth group. This gift enabled me to measure the density of these compounds and to observe a systematic decrease of the size of the ions of rare-earth elements when proceeding from cerium to lutetium, a contraction which explained the extreme kinship of zirconium and hafnium, which are more closely related chemically than any other elements of the periodic system. (When testing the rare earths for radioactivity, making use of Auer von Welsbach's samples, we discovered that samarium emitted α-rays.) In Oslo, V. M. Goldschmidt simultaneously observed the contraction of ionic size, proceeding from one rare earth to the next one and denoted this rare-earth contraction as the "lanthanide contraction." Goldschmidt described his and my work in his posthumously published book *Geochemistry*, a most fascinating reading, like everything that he wrote. V. M. Goldschmidt was one of the most able men I ever met. Endowed with an immense knowledge and a fabulous memory, he was full of fertile ideas.

A few weeks prior to the occupation of Norway, I spent a few days with him at his home on Holmenkollen near Oslo. He predicted the tragic happenings of the coming years, which very few foresaw. He mentioned that his pupil and former assistant Lunde soon would become a "Gauleiter" of Norway. Lunde was later the Minister of propaganda in the Quisling government. Goldschmidt predicted that the Norwegian coast batteries would fail to fire at the invading enemy, which they in fact did with very few exceptions. He was also endowed with much humor. When Quisling came into power, Goldschmidt was imprisoned and all his property seized. Being short of phosphorus fertilizers, the government released him and instructed him to prepare phosphorus from Norwegian minerals. All his property, however, remained confiscated. When German colleagues passed en route to Rjukan, where they had to inspect the heavy-water works, they called on Goldschmidt. He invited them for dinner, encouraging them to eat with the remark : "Please go on eating, gentleman, all you consume is state property."

Tracers in Biology

During the work with hafnium, I continued the tracer work and in 1923 applied radium D and thorium B as tracers in the study of the uptake of lead by bean seedlings and also in the removal of labelled lead by non-

labelled lead from such seedlings. This was the first application of radioactive tracers in biological studies. The following year we extended these studies with my friends Christiansen and Lomholt to the distribution of lead and bismuth in the animal organism.

Potassium is one of the few radioactive elements found in nature outside the members of the disintegration series. We were interested to find out which of the potassium isotopes is radioactive. For this purpose we carried out a partial separation of the potassium isotopes, applying the same method used when separating the isotopes of mercury. A few kilograms of metallic potassium were distilled and a heavy and a light potassium fraction obtained. From the difference in the activity of these samples and the difference in their atomic weight, the mass number of the active isotope could be calculated. Among other instruments that were used to measure the activities of our samples was the first counter built by Geiger in his institute at Kiel. The atomic weight of our sample was determined by Hönigschmid in Munich. From these data it was concluded that the mass number of radioactive potassium is 41.

The first one to draw my attention to the fact that this result was probably wrong was Baxter, when I visited him at Harvard. He had found that, in constrast to all other atomic weight figures determined by Hönigschmid, that of potassium was wrong. Baxter proposed to determine the atomic weight of our potassium samples. From his results it followed that the mass number of radioactive potassium is 40. The two greatest authorities in the field of atomic weight determination thus arrived at different results as to the atomic weight of our potassium samples. To reach a decision, we extracted the small calcium content of an old potassium-rich mica. If ^{41}K were the active isotope, then the calcium isolated should contain ^{41}Ca. Aston could not, however, find any ^{41}Ca in our sample. Thus ^{41}K does not disintegrate and is not radio- active. Baxter was right. A few years later Fermi and collaborators observed the production of an artificial potassium isotope when bombarding potassium with neutrons. We obtained with Miss Hilde Levi Fermi's product by bombarding scandium and also calcium with neutrons. As scandium has only one stable isotope, we could conclude from our investigations that Fermi's radiopotassium has the mass number of 42.

Activation Analysis

Auer von Welsbach was very cautious in giving away his very valuable rare-earth samples, but one day when I was staying with him he was in a generous mood and told me to choose one of his samples, of which

he said he was willing to give me a larger amount. I chose dysprosium without having any special reason to do so. Ten years later, after the discovery of artificial radioactivity, we exposed Auer's dysprosium to slow neutrons and succeeded in producing an exceedingly strongly active radiodysprosium. No element is known that can be actived more intensively than dysprosium and europium. Exposure of Auer's europium to a neutron beam also led to the formation of a very strongly active radioeuropium, while no active gadolinium could be prepared by using radium-beryllium as the source of neutrons. At that time my friend Professor Rolla, of the University of Florence, who prepared a few kilograms of gadolinium oxide, sent me samples of this material which he wished us to analyze for europium by X-ray spectroscopy. We had earlier analyzed several of his samples quantitatively applying secondary X-rays, a method which was worked out in the Freiburg laboratory. Having no access to a Roentgen spectroscope in Copenhagen at this time, we tried together with Miss Hilde Levi to analyze the samples by exposing them to a flux of slow neutrons. All the samples contained some europium.

By preparing standards containing a known amount of pure gadolinium and pure europium, we could arrive at quantitative figures for the europium content of Rolla's samples. This was the start of activation analysis, which has since become an important tool in analytical chemistry. It was possible by this method to determine, for example, the minute amounts of sodium and potassium in a nerve fiber.

Deuterium as a Tracer

Urey's epochal discovery of deuterium took place while I worked in Freiburg. Most kindly he promptly supplied us with some liters of waters containing 0.6 per cent of deuterium oxide. This low heavy-water concentration sufficed to study the interchange of the water molecules between goldfish and the surrounding water and also to determine the water content of the human body, making use of the principle of isotope dilution already introduced a few years earlier (1931) when we determined the lead content of rocks. The mean lifetime of the water molecules in the human body was determined as well. When I returned to Copenhagen in the fall of 1934, August Krogh called on me immediately upon my arrival. He wished to apply labelled water in his permeability studies.

I initially intended, upon return to Copenhagen, to do work with deuterium on similar lines as later published by Schoenheimer and Rittenberg. The possibility of obtaining artificial radioactive isotopes, however, induced me to abandon this plan and to concentrate on the application of radiophosphorus in biological studies.

Radioactive Phosporus

As a neutron source we had only radon-beryllium, later radium-beryllium mixtures, at our disposal. When Niels Bohr celebrated his fiftieth birthday, his friends presented him with 600 milligrams of radium, which he most kindly put at our disposal. With such modest neutron sources, the only tracer of an element of physiological importance which could be produced having sufficient activity was radiophosphorus. We irradiated 10 liters of carbon disulphide from which carrier-free ^{32}P could be easily separated. All our preparations, however, had an activity below 1 μc. The first problem attacked was whether the mineral constituents of the skeleton are renewed or not during life. Labelled phosphate was administered to rats, and the specific activity of their plasma inorganic phosphorus and skeleton apatite phosphorus compared. The comparison indicated a 30 per cent renewal in the course of the first 24 hours. The amount of phosphate involved in this process exceeded twenty times the phosphorus content of the blood. Thus a large part of the phosphorus present in the soft tissues must have been released and applied in the replacement of skeleton phosphorus. This result demonstrated the dynamicity of phosphorus metabolism. These conclusions were published about the same time, in 1935, as the first paper by Schoenheimer and Rittenberg appeared in which they demonstrated the dynamic nature of fat depots. It was followed by a great number of other most illuminating papers in which deuterium, and later heavy nitrogen, was applied as a tracer. Since ^{32}P has a half-life of fourteen days only, happenings through life of a mouse cannot be followed using this tracer. However, applying ^{45}Ca we succeded a few years ago in showing that only one-third of the calcium atoms of the skeleton of the mouse are replaced during life.

The above-mentioned first application of an artificial radioactive isotope as a tracer was followed by our investigation of whether and to what extent the phosphatide molecules of the brain are renewed. These investigations were extended to other organs and to the formation of labelled phosphatides in the chick embryo following the injection of ^{32}P into the fertilized egg. We transfused labelled plasma of a rabbit to a sister rabbit and followed the rate of disappearance of the labelled phosphatide molecules from the circulation of the second rabbit and their accumulation in various organs. The next step was the study of the rate of renewal of the ATP, creatine, and similar molecules, partly in collaboration with Professor Parnas in Lwow, Poland. With Armstrong and also with Krogh and Holst, we studied ^{32}P incorporation in dentine and enamel. In one of the early applications (1937), the penetration of ^{32}P into yeast cells was traced and shown to be an almost one-way process. This investigation was made possible by co-operation with

Linderström-Lang and Olsen at the Carlsberg Laboratory. The first investigations of the uptake of ^{32}P by plants (1936—37) was also carried out in co-operation with them.

In 1940 Professor Hasting, who had formerly visited Copenhagen, invited me to deliver the Dunham Lecture at Harvard University. Denmark was occupied, and messages to the United States could be sent only by the United States Legation in Copenhagen. When I called on the minister asking him to forward a cable to Professor Hasting stating, "I shall be in New York the 21st of June," the Minister remarked, "You 'd better write 'I intend to' ". It was a wise remark, as I did not succeed in getting to the United States and the Dunham Lecture was ultimately delivered by Schoenheimer.

We observed, with Aten, that while phosphate penetrates comparatively slowly into erythrocytes, it is incorporated very rapidly into labile organic acid-soluble molecules. Thus the red corpuscles are a kind of trap, though imperfect, for ^{32}P, a fact which makes it possible to tag red corpuscles with ^{32}P, re-inject these into the circulation, and from the dilution figure calculate the red corpuscle volume of the subject in the course of a day. This method of red corpuscle volume determination found an extended application. The first clinical determinations could be carried out with the minute ^{32}P activities prepared by us by irradiation of carbon disulfide with neutrons emitted by a radium-beryllium source. To investigate the formation of phosphatide or of casein in the milk of the goat, which was the subject of the dissertation of A. H. W. Aten, larger activities were needed. These were prepared by Martin Kamen, put at our disposal by the great kindness of Ernest Lawrence. He supplied us later also with ^{24}Na and ^{42}K. We used these isotopes, among other purposes, to study the rate of interchange of vascular with extravascular ions. We were much impressed by the observation that within the first minute a very large fraction of the sodium ions of the circulation, for example, was replaced by extravascular sodium. Today we know that the exchange-rate values obtained by the tracer method supply minimum figures only.

Max von Laue's and James Frank's Nobel Medals

My work was interrupted for only one day during the enemy occupation of Denmark. When, on the morning of Denmark's occupation, I arrived in the laboratory, I found Bohr worrying about Max von Laue's Nobel medal, which Laue had sent to Copenhagen for safe-keeping. In Hitler's empire it was almost a capital offence to send gold out of the country, and, Laue's name being engraved into the medal, the discovery of this by the invading forces would have had very serious consequences for him. (Three years later the invading army occupied

Bohr's institute.) I suggested that we should bury the medal, but Bohr did not like this idea as the medal might be unearthed. I decided to dissolve it. While the invading forces marched in the streets of Copenhagen, I was busy dissolving Laue's and also James Frank's medals. After the war, the gold was recovered and the Nobel Foundation generously presented Laue and Franck with new Nobel medals.

The Nobel Prize

In December, 1935, on their journey home from Stockholm, where they were presented by King Gustaf V with the Nobel prize, for their fundamental discovery of artificial radioactivity, Frédéric Joliot-Curie and his wife stayed for a while in Copenhagen. It was then that Joliot mentioned that he, his wife, and the third French Nobel laureate, Jean Perrin, proposed me for the Nobel prize and also that they failed to obtain the adherence of the Paris Academy to their proposal — the celtium-hafnium controversy was not yet forgotten. During the war Niels Bohr with his extreme kindness remarked to one of his friends that one of the numerous disturbances created by the war was that I could not receive the Nobel prize. The shocking refusal of the acceptance of the prize by Domagk, Butenandt, and Kuhn at the order of their ruler made the Swedish Academy of Sciences reluctant to distribute further prizes during the war. In 1944 the Academy decided, however, to award me the prize for 1943. With the war going on, no festivities were held, and the prize, contrary to the usual custom, was handed over to me in a meeting of the Academy of Sciences by the president.

Radioactive Tracers in Radiobiology

In 1940 we got interested, with L. v. Hahn, in the formation rate of desoxyribonucleic acid, DNA. While the incorporation of ^{32}P, for example, into adenosintriphosphate of the growing liver indicates mainly renewal of these molecules and not an additional formation, the incorporation into desoxyribonucleic acid indicates the latter to at least a very large extent.

By investigation of the effect of ionizing radiation on the incorporation of ^{32}P into DNA, it should thus be possible to find out if irradiation blocks DNA formation. Together with Professor Hans von Euler, we studied in Stockholm the incorporation of ^{32}P into the DNA of the Jensen sarcoma of rats and found in the investigated 100 rats exposed to Roentgen rays a marked depression of ^{32}P incorporation, and thus a marked depression in the rate of formation of DNA. Similar results were obtained when investigating ^{32}P incorporation into the DNA in the various organs of growing rats. Indirect radiation effects were

observed by us as well. These were among the first application of radioactive tracers in radiobiological studies. Our joint investigations, among others, were extended to the determination of the number of fertilizing asp pollen, the atoms of which can be located in a seed. The incorporation of ^{32}P into DNA of the nucleated erythrocytes of the hen was found, in collaboration with Ottesen about the same time, to be quantitatively conserved during the lifetime of the erythrocytes, which enabled us to measure the life-cycle of the red corpuscles of the hen.

Prior to and during the war I saw a lot of August Krogh, famous physiologist and a man of great kindness, to whom I was much indebted. While staying in Stockholm, he wrote down a detailed program of further permeability studies in which radioactive tracers would have to be applied. It is much to be deplored that he could not witness the great further success of his eminent pupil Ussing in this field.

Radioactive Carbon

My chief activities since 1943 have been in Stockholm and, for some years after the war, in Copenhagen too. During the last years I have been attending solely to my laboratory in Stockholm. I extended the radiation studies to the measurement of ^{14}C incorporation into DNA in the organs of growing mice, which was found to be depressed in contrast to incorporation into proteins. My colleague Forssberg and I studied the effect of irradiation on bicarbonate, glucose, and fatty acid metabolism and other problems, applying ^{14}C as a tracer. These studies, among others, led to the discovery of a fatty acid fraction of the liver having a very rapid turnover rate. For the last years we have been interested in physiological and clinical problems of iron metabolism.

In 1953 I had the privilege to deliver the Aschoff Memorial Lecture, which is given each year in the University of Freiburg to commemorate the great pathologist. Aschoff was not only one of the great pathologists of this century but a man of great wisdom and vision. The British pathologist Robert Muir wrote in his obituary- note on Aschoff, published during the war, "I think one may say that in the period since Virchow's time, he has been the outstanding figure." Aschoff showed some interest in our early work with lead and was quite enthusiastic about the determination of the volume of the body water by applying heavy water as an indicator, which was the first clinical application of isotopic tracers. In my Lecture I mentioned that our investigations had led us to the conclusion, not unanimously accepted by the audience, that the formation of haemoglobin is not radiosensitive, that so long as erythropoetic cells with an incomplete haemoglobin content are present in the bone marrow, even if the organism is exposed to Roentgen radiation, additional hemoglobin is laid down in these cells. Since then this conclusion has

been fully corroborated by work carried out in our laboratory, and especially by the beautiful work carried out in Oxford by Lajtha and his associates.

When we started with Paneth in the first days of 1913 to apply radium D as a tracer of lead, the word "isotope" was not yet coined. Groups of radioactive substances such as mesothorium and radium, or ionium and thorium, were denoted by Soddy as "chemically inseparable elements". Much has happened since those days!

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1. THE SOLUBILITY OF LEAD SULPHIDE AND LEAD CHROMATE

George Hevesy and Fritz Paneth
From the Institute of Radium Research of the Vienna Academy of Sciences

The fourth decay product of radium emanation, RaD, is known to exhibit all the chemical reactions of lead; if RaD is mixed with lead or lead salts it cannot be separated from the lead by any chemical or physical method[1] and if complete mixing of the two substances has taken place then the same concentration ratio is maintained whatever amount of lead is withdrawn from the solution. Since RaD can be determined in much smaller amounts, owing to its radioactivity, than lead, it may be employed for the qualitative and quantitative estimation of lead to which it has been added; the RaD is an indicator of the lead.

The lower limit for the qualitative detectability of lead in its most sensitive microchemical reaction[2] (precipitation of $K_2PbCu(NO_2)_6$) amounts to 3×10^{-9} gm; the limit for quantitative determination lies considerably higher and varies with the particular problem; for example, the solubility of lead carbonate could be obtained from determinations of the conductance but Kohlrausch[3] was able only to make an approximate estimate for lead chromate in this way. With the aid of RaD as a tracer these solubilities can easily be determined directly; an amount as small as 10^{-10} gm RaD may be measured, by means of an ordinary and not particularly sensitive electroscope, if one is content to measure the β-radiation of RaE which comes to equilibrium with the RaD after a few weeks. By awaiting the formation of a quantity of RaF sufficient for calculating the equilibrium amount, it is possible to determine quantitatively even 10^{-12} gm of RaD by means of the α-radiation. In radiolead from pitchblende there is about 10^{-7} gm of RaD per gm of lead and thus 1 mgm of radiolead can be detected with the aid of its β-radiation; since much smaller orders of magnitude are involved

[1] A review of relevant experiments is contained in the paper by F. Paneth and G. Hevesy in *Monatsh. Chem.* **42,** 1 (1913).
[2] J. Emich, *Lehrbuch d. Mikrochemie* p. 80 (1911).
[3] F. Kohlrausch, *Z. phys. Chem.* **64,** 159 (1908).

in the solubilities discussed above we must therefore prepare artificially radiolead by the addition of relatively large amounts of radium-D to lead nitrate.

1. DETERMINATION OF THE SOLUBILITY OF LEAD CHROMATE

About 0.2 c of emanation was allowed to decay in a closed flask over distilled water and the solution thus obtained, containing about 10^{-6} gm RaD in water, was added to a solution of approximately 10 mgm $PbCl_2$ in water. The lead was then quantitatively precipitated with potassium chromate, filtered, washed from the filter into a stoppered bottle and shaken with about 100 cm^3 of distilled water in a thermostat at 25° for a period of 24 hr. The mixture was immediately filtered, the first portion of the filtrate being rejected because of a possible change in its concentration as a result of adsorption on the filter, and 70 cm^3 of the remaining filtrate was evaporated to dryness on a watch-glass-shaped nickel tray over the water bath. When equilibrium had been established between the RaD and RaE the activity on the tray was measured.

The calculation was done as follows: 1 cm^3 of the RaD solution used for labelling the lead showed (also after establishment of equilibrium) a β-activity of 16.90 arbitrary units and, therefore, the whole solution, amounting to 120 cm^3, contained 2030 units. This activity had been distributed on 9.69 mgm of lead chloride or 11.35 mgm of lead chromate and thus one arbitrary unit of RaD was associated with $11.35/2030 = 0.00559$ mgm lead chromate. The 70 cm^3 of solution which had been evaporated had deposited an activity of 0.15 units on the tray and thus $0.15 \times 0.00559 = 0.000839$ mgm of lead chromate must be on the tray. Hence, the solubility of lead chromate at 25° C is calculated to be $1000 \times 0.000839/70 = 0.012$ mgm/l.

A second experiment with the same solid phase also gave 1.2×10^{-5} gm/l. The first experiments, carried out with smaller amounts of RaD and accordingly with a much lower accuracy, yielded values which varied between 3×10^{-5} and 6×10^{-5} gm. Lead chromate is therefore the most sparingly soluble lead salt; only the solubility of lead phosphate is of the same order of magnitude.

Apart from a rough estimate by F. KOHLRAUSCH[1] based on a measurement of conductance of the saturated lead chromate solution, there are no data available on the solubility of lead chromate; KOHLRAUSCH estimates the solubility as 10^{-4} gm/l.

[1] F. KOHLRAUSCH, *Z. phys. Chem.* **64**, 159 (1908).

2. DETERMINATION OF THE SOLUBILITY OF LEAD SULPHIDE

For these experiments 9.69 mgm of lead chloride (8.36 mgm in terms of sulphide) were labelled with 140 cm^3 of another solution of RaD which contained 66.2 arbitrary units per cm^3. The lead was then quantitatively precipitated at the boil with a hot solution of Na_2S, the PbS was filtered off, washed and shaken with distilled water as described in the case of lead chromate. The filtrate, the first part of which was again rejected, was completely clear and colourless; it contained 415 arbitrary units of RaD per litre. In this instance one arbitrary unit corresponds to $8.36/140 \times 66.2 = 9.0 \times 10^{-4}$ mgm of lead sulphide, and thus 1 l. of solution at 25° C contained $415 \times 9.0 \times 10^{-4} = 0.37$ mgm or 3.7×10^{-4} gm. The same value was obtained after filtering the solution once again; other experiments yielded the values of 300 and 320 arbitrary units per litre, i.e. 2.70 and 2.88×10^{-4} gm lead sulphide per litre.

A part of the lead present in the solution probably occurs as hydroxide, owing to hydrolysis, instead of sulphide, as suggested by O. Weigel[2]. The very weak turbidity obtained when the completely clear saturated solution, prepared by shaking water with PbS, is treated with a stream of H_2S supports this view. We have therefore determined the solubility of PbS in water saturated with H_2S; the solution from which the PbS is precipitated cannot be used directly for determining the solubility since a portion of the PbS passes as a colloid through the filter; the once-filtered PbS, on the contrary, is already freed from the small particles passing through the filter and these do not recur when the solution is shaken with distilled or H_2S-saturated water. In the solution which is saturated with H_2S and PbS, the concentration of H_2S is about one thousand times that of the PbS. The solubility of the latter is less than the value obtained in distilled water; calculated on the basis of 1 l., the arbitrary activity amounted to 148 and 173 and hence the amount dissolved was 1.33 and 1.56×10^{-4} gm, respectively. It is not possible to decide with certainty whether there is a decrease in solubility due to an increase of the S ion concentration or due to prevention of hydrolysis; the first case, however, is improbable since the decrease in solubility is only very slight in proportion to the high concentration of H_2S. In analytical practice, moreover, this problem need not be considered; it is only of interest to know the amount of PbS which is present in solution in a clear filtrate; our experiments give an average value for this of 3×10^{-4} gm in the absence of H_2S and 1.5×10^{-4} in a solution saturated with H_2S. If the filtrate runs turbid through the filter, it is evident that the amount of PbS will be greater. In one instance we observed 1—2 mgm/l.

[2] O. Weigel, *Z. phys. Chem.* **55,** 293 (1907).

W. Biltz[1] determined the solubility of PbS by means of an ultramicroscopic method: When two equivalent solutions which produce a precipitate are mixed in a series of experiments at increasing dilution and the mixture obtained is observed with an ultramicroscope it is noted that, beyond the limit of macroscopic differences, the number of suspended particles of the precipitate becomes steadily less until, at a certain dilution, the mixture appears to be empty or no longer different from its components. This limiting value for the disappearance of the undissolved excess corresponds to the solubility of the substance produced, i.e. lead sulphide. Biltz finds a value of 1.3 mgm/l. for the solubility of lead sulphide at room temperature. He remarks that the determination is made more difficult, in the case of sulphides, because they form colloidal solutions which are almost optically transparent at a high dilution ; separate particles can of course be produced by adding salting-out electrolytes with dissimilar ions but at the same time this may cause an increase in solubility. The solubility determined by the ultramicroscopic method is therefore probably rather too large. Correspondingly, O. Weigel[1] found that 0.86 mgm of freshly precipitated PbS dissolved in 1 l. by calculating the solubility of PbS from the conductance on the assumption that all the PbS going into solution is hydrolysed. Freshly precipitated PbS, however, undergoes a transformation and after about 20 hr have elapsed the solubility amounts only to about 0.43 mgm/l. The PbS used in our experiments was already transformed and the solubility of 3×10^{-4} gm in 1 l. which we found agrees very well with Weigel's value[2].

RaD is not the only radioelement which can serve as an indicator for lead ; careful studies by Fleck[3] demonstrate that thorium-B, radium-B and actinium-B also cannot be separated from lead. The last two cannot indeed be considered for our purposes but thorium-B, with its half-life of 10.6 hr, might well be applied with success as an indicator for lead.

Besides lead, we know of two other elements with which a radioelement can be used in practice as an indicator, viz. bismuth and thorium. The former can be labelled with thorium-C or preferably with RaE,[4] while the latter may be labelled with uranium-X, radiothorium or, best of all, ionium[5].

[1] W. Biltz, *Z. phys. Chem.* **58,** 288 (1907).

[1] O. Weigel, *Z. phys. Chem.* **55,** 293 (1907).

[2] I. Bernfeld, *Z. phys. Chem.* **25** (1898) considers the PbS electrode to be a reversible electrode of the second kind and calculates the lead ion concentration to be 10^{-4} at the PbS electrode at one atmosphere pressure of hydrogen sulphide from the electromotive force of the cell Pb | 1N $Pb(NO_3)_2$ | 1N NaHS | PbS.

[3] A. Fleck, *Proc. Chem. Soc.* **29,** 7 (1913).

[4] A. Fleck, *Proc. Chem. Soc.* **29,** 7 (1913).

[5] F. Soddy, *Radiochemistry.* London (1911).

An advantage of the indicator method is that, irrespective of impurities, only the amount of the labelled element is measured, whereas in other very highly developed microanalytical methods of determination, e.g. by employing microweighing, there is always the danger of co-determining invisible impurities. Apart from this, the sensitivity of the radioactive indicator methods is indeed significantly greater and, assuming the availability of adequate amounts of the radioactive substance, can be increased almost without limit.

Summary

The solubility of lead chromate at 25° in pure water has been determined as 1.2×10^{-5} gm/l.; for lead sulphide at 25° in pure water and in H_2S-saturated water the values were 3×10^{-4} and 1.5×10^{4} gm/l. respectively; RaD was used as a tracer for lead.

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2. PLATINUM BLACK

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For the preparation of platinum black, which is used in hydrogen electrodes and for other purposes, the electrolysis of hydrochloric acid solutions of platinum containing lead acetate is employed. The question then arose as to whether the presence of lead in the solution is essential to the preparation of good platinum black and, if so, as to the part played by the lead. To obtain an answer to the first question, we have electrolysed both hydrochloric acid solutions containing only platinum chloride and others containing also small quantities of lead. It was shown that platinum black cannot be obtained successfully by the electrolysis of a solution containing only platinum. On the contrary, a grey or light brown deposit is always obtained. On the other hand, the preparation of platinum black is accomplished from solutions which contain a corresponding amount of other heavy metals in place of lead. After this observation we proceeded to study whether lead is carried into the deposit when a solution containing lead is electrolysed and, if so, in what amount and form.

DETERMINATION OF THE LEAD CONTENT OF PLATINUM BLACK

Since the detection of small quantities of lead in platinum is very tedious we have made use of a radioactive tracer method. A known amount of lead acetate labelled with thorium-B was added to the platinum chloride solution and the lead content of the platinum black deposited on platinum electrodes having a surface area of 15.07 cm^2, at a current density of 10 mA/cm^2, was determined by measuring the intensity of the α-radiation emitted by the deposit. The amount of lead was calculated from this intensity measurement as follows: From the same radioactive lead acetate solution, of which a known volume was added to the platinum chloride solution, lead peroxide was precipitated (see below) after adding nitric acid and a further amount of inactive lead acetate and the α-radiation of this precipitate was compared with

that of the platinum black. Now if care is taken that the thickness of the deposits attains the range of the α-radiation in the material, which is 12.8 μ in lead and 30.6 μ in lead peroxide, then the activity will provide a simple measure of the lead content. Denoting the activity of the platinum black electrode by S_1, that of the lead peroxide electrode of the same size by S_2, the density of platinum (21.3) by d_1, the density of lead peroxide (8.9) by d_2, the range of α-radiation in platinum (12.8 μ) by R_1, the range of α-radiation in lead peroxide (30.6 μ) by R_2, the number of grammes of lead in 1 cm^3 of the active lead acetate solution by p and the number of grammes of lead in the 2.5% lead acetate solution by P, then the required lead content of the platinum black, x, expressed as a percentage, is given by

$$x = S_1 p R_2 d_2(\text{at. wt. of Pb})100/S_2 p P R_1 d_1(\text{mol. wt. of } PbO_2)$$

Therefore p was chosen to differ from P because it had been found preferable to produce the lead peroxide deposit from a solution with a lead content higher than that for the platinum black coating. The lead content of the platinum black, as determined, is found in the Figures in Table 1. It may be seen that the lead content of the platinum black rises sharply with increasing lead acetate content of the platinum chloride solution.

Table 1. — Lead Content of Platinum Black as a Function of the Lead Acetate Content of the Electrolysed 0.2 N HCl Solution Containing 3% $PtCl_4$

Lead Acetate Content of the Solution (%)	Lead Content of the Platinum Black (%)
1.22	0.035
1.34	0.815
1.44	1.5
1.9	7.1

In order to decide whether the lead found in platinum black is present in solid solution or not we have compared the line distances obtained on Debye-Scherrer diagrams for different platinum black samples with those of pure platinum. The difference, as is evident from Table 2, was shown to be vanishingly small and therefore it must be assumed that the large majority of the lead occurring in platinum black is not present in the form of a solid solution. The measured line distances underwent a considerable increase when the sample was heated. Thus, the second sample recorded in Table 2 showed, after heating for 16 hr in a vacuum to 500°C, a line distance of 124.7 mm ; after 44 hr heating at 625 the distance was 125.2 mm.

Table 2. — Line Distance of the (422) Interference of Platinum Black

Lead content of the platinum (%)	Line distance before heating (mm)	Line distance after heating in vacuum (mm)
0	124.3	124.6 (500°)
1.5	124.4	125.2 (625°)
7.1	124.1	125.7 (625°)

Heating to still higher temperatures resulted in a considerable evaporation of lead, as shown in Table 3. With regard to the values in Table 2, it should be mentioned that it was not possible to assess the line distance in the case of platinum grey (lead-free platinum deposit) with sufficient accuracy. The number 124.3 in the second column thus refers to platinum wire whereas the corresponding value in the third column was indeed obtained from platinum grey. After heating, the platinum grey did of course yield lines of adequate sharpness. The exposures for the Debye-Scherrer diagrams were obtained with the aid of the precision camera described by Sachs and Weerts[1]; platinum wires 4 mm thick and coated with platinum black were used for the exposures. A Metalix tube with a copper *anti*-cathode was used as the source of radiation and was operated for as long as 13 hr at 45 kV and 20 mA. The lead content present as a solid solution was calculated by means of Vegard's law, according to which the lattice constant of the solid solution is

$$a = (3.905c_1 + 4.93c_2)/100$$

where a is the length of the side of the unit cell of the alloy, c_1 is the number of atoms per cent of platinum and c_2 for lead. The calculation showed that, of a total of 1.5 per cent lead, only 0.2 per cent was present in solid solution after heating (Table 2) and of the 7.1 per cent lead in another sample only 0.3 per cent was similarly in solid solution.

It is hoped to study in more detail, by means of radiographs of platinum black containing thorium-B, the distribution of lead in platinum.

THE EFFECT OF HEATING ON THE LEAD CONTENT OF PLATINUM BLACK

It has already been mentioned that considerable amounts of lead evaporated when platinum black was heated at higher temperatures. In a more detailed study of this point the alpha activity of platinum black,

[1] Sachs and Weerts, *Z. Phys.* **60,** 481 (1930).

obtained by the electrolysis of solutions containing lead acetate, labelled with thorium B, was determined before and after heating. The results of this experiment are seen in Table 3.

Table 3. — Effect of Heating for 16 hr in a Vacuum on the Lead-Content of Platinum Black originally Containing 1.5% Lead

Temperature (°C)	600—610	630—650	715—725
Loss of lead calculated from the decrease in α-activity (%)	2	53	85

The loss of lead is not entirely due to evaporation but partly also to diffusion of the lead contained in the platinum black into the platinum foil on which the coating was deposited.

Differentiation between the loss by evaporation and diffusion is possible by making use of the γ-radiation instead of the α-radiation for making the comparison; whereas the amount of lead removed by diffusion weakens the α-radiation to the same extent as does the lead disappearing through evaporation, this is is not the case when the γ-activity is measured. For example, the decrease in γ-radiation after 16 hr heating at 685 to 700°C amounted to only 42 per cent and thus considerably less than the decrease in α-radiation.

THE QUALITY OF THE VARIOUS SAMPLES OF PLATINUM BLACK

An attempt was next made to measure the easily traced adsorption of thorium B and thorium C from solutions of these radio-elements with a view to assessing the quality of the platinum black. Yet great difficulty was encountered in obtaining reproducible results. This method was therefore relinquished. It was then thought that a simple measure of the quality of the various platinum black coatings could be obtained by preparing hydrogen electrodes from the various platinum black samples and comparing their potentials. It was shown, however, that the potential of all the hydrogen electrodes prepared in this way was always the same within the experimental error of about 1 mV. We then changed over to determining how strongly the various samples of platinum black could be polarized with the same cathodic loading and to making use of the difference in polarizability as a measure of the quality of platinum black. The polarization was performed in N sulphuric acid solution with a current density of 20 mA/cm^2 at room temperature for a period of 45 min; the area of one side of the electrode amounted to 1 cm^2. The polariz-

ation potential was measured by using a normal hydrogen electrode. A constant value of the polarization potential was established after about 30 min. The result of the measurements is evident in Table 4.

TABLE 4. — CATHODIC POLARIZABILITY OF PLATINUM DEPOSITS OBTAINED FROM SOLUTIONS WITH DIFFERENT LEAD CONTENTS. POLARIZATION CURRENT DENSITY 20 mA/cm²

Lead content of the electrode (%)	7.1	1.5	0.15	0.035	0
Polarization potential (mV)	84.4	77.5	81.0	97.9	103.7

The least polarizable and, therefore, the one of highest quality is platinum black with a lead content of 1.5 per cent, and it is interesting to notice that this sample of platinum black is identical with that obtained by electrolysing a solution containing 1 part of platinum chloride and 0.008 parts of lead acetate in 30 parts of water, and that LUMMER and KURLBAUM a long time ago used the electrolysis of a solution of this composition for preparing platinum black. This set of directions is also included in the *Textbook of Practical Physics* by KOHLRAUSCH and other similar works.

Attempts were then made to heat the electrodes before they were polarized. In all instances the heating spoiled the quality of the platinum black. After heating the electrode containing 1.5 per cent lead for 16 hr at about 610°C the polarization potential rose from 77.5 to 88.3 mV, and after 16 hr heating at about 700° it became 183 mV.

Changing the current density from 10 to 30mA/cm² when preparing the platinum black had no detectable effect on the quality.

CONNEXION BETWEEN THE PARTICLE SIZE AND QUALITY OF PLATINUM BLACK

The particle size of the platinum black was determined from the half breadth of the X-ray lines in accordance with BRILL's method[1]. The Debye-Scherrer camera used for this purpose had a diameter of 5.73 cm. The diameter of the platinum wire covered with platinum black was 0.34 mm. Lines of the (220) and (311) faces were used for the investigation. The results of this study are shown by the data in Table 5.

[1] R. BRILL, *Kolloid Z.* **55,** 164 (1931); *Z. Krist.* **74,** (147 (1930).

TABLE 5. — DEPENDENCE OF THE PARTICLE SIZE OF PLATINUM BLACK ON ITS LEAD CONTENT

Lead content of platinum (%)	Particle size (Å)	
	Calculated from the (220) line	Calculated from the (311) line
7.1	62	64
1.5	81	75
0.15	68	68
0.035	58	
0	61	57

It is evident from this table that the platinum black sample which has been found to have the best quality is distinguished by having the largest particle size.

PREPARATION OF PLATINUM BLACK FROM SOLUTIONS CONTAINING GOLD

It is evident from Table 6 that platinum black of good quality was prepared also from platinum chloride solutions which contained gold instead of lead and was deposited at a current of 30 mA/cm².

TABLE 6. — CATHODIC POLARIZABILITY OF PLATINUM DEPOSITS PREPARED FROM SOLUTIONS HAVING VARIOUS GOLD CONTENTS. POLARIZATION CURRENT DENSITY 20 mA/cm². ELECTROLYTE 1 N H_2SO_4

Platinum content of the solution from which the platinum black was obtained (%)	Gold content of the solution (%)	Polarization potential (mV)
0.14	0.9	76.9
1.8	0.1	78.7
1.8	0.01	77.7
1.8	0.0001	86.4

We have also prepared platinum black from platinum chloride solutions which contained thallium, cadmium or zinc in place of lead. Whereas thallium can substantially replace lead as far as the appearance of the deposit is concerned, the behaviour with cadmium is different inasmuch as a solution which contained about 0.02 per cent cadmium chloride yielded a fine black deposit while the electrolysis of solutions which contained only about 0.01 per cent cadmium chloride yielded a grey deposit instead of platinum black. Electrolysis of platinum chloride solutions containing zinc yielded a grey deposit in all cases. We then

attempted to electrolyse hydrochloric acid solutions of pure platinum chloride at a high current density, i. e. at 100 mA/cm² and above. With this heavy loading it was no longer possible to obtain an adherent deposit. The deposited platinum powder fell into the solution, had a black-grey appearance and was extraordinarily fine grained.

Summary

The platinum black obtained from platinum chloride solutions containing lead in accordance with the directions of LUMMER and KURLBAUM, contains considerable amounts (1.5 per cent) of lead. Variation of the lead content of the platinum black with the lead content of the solution subjected to electrolysis was observed.

The electrolysis of a solution containing 1.9 per cent of lead acetate yields a platinum black which contains 7 per cent of lead. By assuming the validity of VEGARD's additive law for the lattice dimensions it is found that the greater part of the lead present in platinum black does not occur in solid solution.

Of the various samples of platinum black the one prepared in accordance with the directions of LUMMER and KURLBAUM was the least electrolytically polarizable and thus the most perfect. A determination of the particle size of the platinum black samples by means of the half-width of the X-ray interferences resulted in the fact that the best platinum black sample had the largest particle size.

Platinum black was also prepared from platinum chloride solutions which contained other added metals instead of lead.

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3. LEAD CONTENT OF ROCKS

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In recent years various geochemical problems have arisen which make it important that our scanty knowledge of the lead content of rocks should be amplified and made more precise. To this end we have determined the lead in a series of samples, representing in all about 220 rocks, some of which we owe to the kindness of Prof. Arthur Holmes of the University of Durham.

The sample to be analysed was brought into solution ; silver sulphate was added and the silver and lead present in the solution were simultaneously precipitated as sulphide. The precipitate was them brought into solution and the lead deposited electrolytically as peroxide. That the deposit was actually lead peroxide was confirmed by a colorimetric test, tetramethyl-diamino-diphenylmethane being added to the solution of the deposit. To ascertain that the total lead content was actually recovered, we used the method of radioactive indicators. We added to the rock sample a known amount of the lead isotope radium D, prepared from radium emanation, and checked the yield obtained by measuring the activity of the lead peroxide deposit. As the purest chemicals commercially obtainable were found to contain quantities of lead that would have influenced our results, all the chemicals used were first purified from lead. Moreover, every precaution was taken to avoid contamination of the samples by dust which might have contained traces of lead. The results obtained are listed in Table 1.

The average value found is 16×10^{-6} gm lead per gm rock, a somewhat larger value than that given by Clarke and Steiger[1] who found 7.5×10^{-6} gm per gm rock. As shown in the communication that follows, the amount of lead accumulated in the rocks since the solidification of the earth's crust (as a result of the decay of uranium and thorium) is very much smaller. Thus, as between the atomic weights of rock-lead and ore-lead we have in most cases to expect differences only in the

[1] Clarke and Steiger, *J. Wash. Acad. Sci.* **4**, 58 (1914).

TABLE I. — LEAD CONTENT OF IGNEOUS ROCKS

Rock Types	gm Lead per gm Rock
Basalt, Giant's Causeway	4×10^{-6}
Gabbros and related types (composite of 67 samples)	5×10^{-6}
Essexites and related types (composite of 40 samples)	10×10^{-6}
Shonkinites (average of 2 samples)	10×10^{-6}
Soda-granites and soda-syenites (composite of 26 samples)	11×10^{-6}
Potash-granites and potash-syenites (composite of 24 samples)	14×10^{-6}
Amphibolite, inclusion in Kimberlite, Wesselton Mine, S. Africa	15×10^{-6}
Kimberlite ('basaltic' type). Dyke from 1350-foot level, Dutoitspan Mine	16×10^{-6}
Lherzolite, Baltimore, Maryland	19×10^{-6}
Granitic rocks (composite of 58 samples of widely different localities)	30×10^{-6}
Dunite, Jackson Co., North Carolina	42×10^{-6}

second decimal place. That the ore-lead must have been formed in the ancestral sun, or during the events that attended the birth of the solar system, was already pointed out some years ago by Prof. HOLMES[1].

We conclude from the above determinations that the greater part of rock-lead is also of the same origin. Although acid rocks, which have a relatively high uranium and thorium content, are found to contain more lead than basic rocks, this difference is not to be interpreted as an argument in favour of the radioactive origin of the whole of the lead in rocks, but an expression of the fact that lead, like uranium and thorium, shows a marked affinity to siliceous magmas.

It is of interest to compare the lead content of basic and ultrabasic rocks with that of meteorites as determined by NODDACK[2]. The lead content of stony meteorites is near that of basalt and average gabbro, and is markedly lower than that of terrestrial ultrabasic rocks. The lead content of iron meteorites, as confirmed in this laboratory, is about ten times greater than that of stony meteorites, while that of troilite (the high lead content of which was predicted by Prof. V. M. GOLDSCHMIDT) is more than a hundred times greater (700×10^{-6}). These results show that when the earth was formed the silicate shell received only a modest

[1] HOLMES, *Nature* **117**, 482 (1926).
[2] NODDACK, *Die Naturwiss.* **18**, 761 (1930).

TABLE II. — LEAD CONTENT OF BASIC AND ULTRABASIC ROCKS AND OF METEORITES

	gm per gm Rock
Gabbros (average)	5×10^{-6}
Kimberlite	$16 \quad 10^{-6}$
Lherzolite	19×10^{-6}
Stony meteorites (average)	5×10^{-6}
Iron meteorites (average)	50×10^{-6}

share of the total lead available for partition, and that this uneven distribution has so far been compensated only slightly by the formation of lead from radioactive decay.

Comment on papers 1, 2, 3

Radioactive tracers are very often applied in the solution of problems which can only be solved by making use of this device. Often this method is, however, applied not as a necessity but for the sake of convenience, for facilitating the solution of problems which can be solved by other methods as well, though more tediously. An example of the latter is the determination of the lead content of platinum black described in paper 2. By making use of labelled lead the analysis and the behaviour of platinum black under the effect of heat, of electrolytic polarization and so on could be carried out without even dissolving the sample

The determination of the solubility of lead sulphide and chromate in water discussed in papers is a border case. The writer of the well-known text-book on physical measurements, Kohlrausch, succeeded in calculating the solubility of lead carbonate from the electrolytic conductivity data, but he arrived in the case of lead chromate at a rough estimate of its solubility only.

In the determination of the lead content of rocks by making use of isotope dilution control an isotope of lead is necessarily to be used. This investigation was the first application of the isotope dilution method. Being interested in the abundance of the elements, we determined various constituents of an artificial air crust sample prepared by my colleague, the well-known mineralogist Schneiderhöhn, who disposed over a very extended collection of rock and mineral samples. We usually applied the method of analysis of X-ray spectroscopy using a secondary X-radiation method worked out in the Freiburg laboratory in which these investigations were carried out.

Knowing the intensity ratio of two very closely situated X-ray lines, for example that of HfL_1 and $Lu\beta_2$, by adding to a pulverized sample to be analysed a known amount of LuO_2 we can, after taking an X-ray spectrum, calculate from the intensity ratio of the 2 above-mentioned lines the unknown hafnium content of the sample. When the X-ray spectrum is excited by cathode rays the sample gets hot and sputters easily; for this reason a continuous X-ray spectrum is first produced on a metallic tungsten surface and the sample irradiated by this continuous röntgen radiation. Heating of the sample and consequent sputtering is now avoided. The application of the method in the above-mentioned case assumes the absence of significant amounts of the rare lutetium in the sample investigated.

The sensitivity of this method (in the twenties of this century, when we applied it) did not suffice to determine the very small amounts of lead present in rock samples and correspondingly we embarked on a chemical determination of lead controlled by making use of isotope dilution. This device, discussed further on p. 96, proved to be a very useful one in chemical analysis.

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4. THE ACTION OF NEUTRONS ON THE RARE EARTH ELEMENTS

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The action of neutrons on the rare earth elements can be followed up in two ways : by investigating the radioactivity induced in these elements under neutron bombardment, and by observing their absorbing power for a beam of slow neutrons. In this paper both these lines of attack will be discussed for the rare earth group and for yttrium and scandium.

ARTIFICIAL RADIOACTIVITY OF THE RARE EARTH ELEMENTS

The artificial radioactivity of some of the rare earth elements was investigated by Amaldi, D'Agostino, Fermi, Pontecorvo, Rasetti and Segrè (1), others were investigated by ourselves (2) by Sugden (3) by Marsh and Sugden (4) by McLennan and Rann (5) and by E. Rona (6). The neutrons used were produced by the action on beryllium of the α-rays from radium emanation and were in many cases slowed down by inserting layers of paraffin 10—20 cm thick in the path of neutrons ; a Geiger—Müller counter was used to measure the activities obtained.

Scandium

A sample of scandium oxide prepared by Prof. Sterba-Böhm and kindly presented to us by Prof. Hönigschmid, who used the preparation in determining the atomic weight of scandium, was activated for a few days using an emanation-beryllium source of 200—300 MC. The oxide was then dissolved in dilute hydrochloric acid and 100—150 mgm sodium chloride as a carrier of ^{42}K (cf. p. 48) and the same amount of calcium oxide were added. The filtrate obtained after precipitation with carbonate-free ammonia was treated with oxalic acid and the calcium oxalate-formed was removed. The sodium chloride which had been added was recovered, after the removal of the ammonium chloride content of the last filtrate, by evaporation and ignition. The activities of the three

fractions, those of scandium oxide, sodium chloride, and calcium oxalate, were then determined. The two first mentioned preparations were found to be active, the activity of the scandium oxide decaying very slowly and that of the sodium chloride fraction having a half-life of 10 to 16 hours. The activities are due to the formation of $^{46}_{21}$Sc and $^{42}_{19}$K respectively; the reactions leading to these products are

$$^{45}_{21}\mathrm{Sc} + {}^{1}_{0}\mathrm{n} = {}^{46}_{21}\mathrm{Sc}$$

and

$$^{45}_{21}\mathrm{Sc} + {}^{1}_{0}\mathrm{n} = {}^{42}_{19}\mathrm{K} + {}^{4}_{2}\alpha\,.$$

The mass numbers occuring in these equations follow from the fact that scandium has only one stable isotope, ^{45}Sc. The calcium oxalate investigated was inactive; we are thus unable to find any evidence for the reaction $^{45}_{21}\mathrm{Sc} + {}^{1}_{0}\mathrm{n} = {}^{45}_{20}\mathrm{Ca} + {}^{1}_{1}\mathrm{H}$ which possibly takes place also. The activity which cannot be separated from scandium is presumably due to $^{46}_{21}$Sc; most of this activity decays with a period of about two months

While $^{42}_{19}$K emits hard β-rays having a half value thickness of 0.19 gm/cm^2 Al, $^{46}_{21}$Sc emits soft β-rays with a half value thickness of 0.01 gm/cm^2 Al.

Yttrium

We investigated (2) samples of yttrium oxide kindly given us by the late Baron Auer v. Welsbach, by Prof. Prandtl, and by Prof. Rolla. The two first named preparations were used some time ago by Hönigschmid to determine the atomic weight of yttrium and investigated by one of us on that occasion by X-ray spectroscopy. While the investigation of Baron Auer's preparation revealed the presence of some dysprosium, that of Prandtl was found to be of the highest purity. The great purity of this preparation and of that of Rolla was also shown by their behaviour under neutron bombardment: No initial decay with the period of dysprosium (2.5 h.) could be observed, the sole period being one of 70 h., which we found to be the period of decay of yttrium. Auer's preparation decayed initially with a half-life of 2.5 h., which was obviously that of dysprosium; but afterwards it showed a 70 h. period like the other preparations. The molecular volumes of corresponding compounds of yttrium and dysprosium are only very slightly different*, so these elements are unusually closely related chemi-

* The volumes of the octahydrosulfates differ by less than 0.8% (G. v. Hevesy, *Z. anorg. Ch.* **147,** 217; **150,** 68 (1925) and the ionic radii by about the same amount (V. M. Goldschmidt, Ullrich and Barth, *Oslo. Acad. Proc.* Nr. 5 (1925).

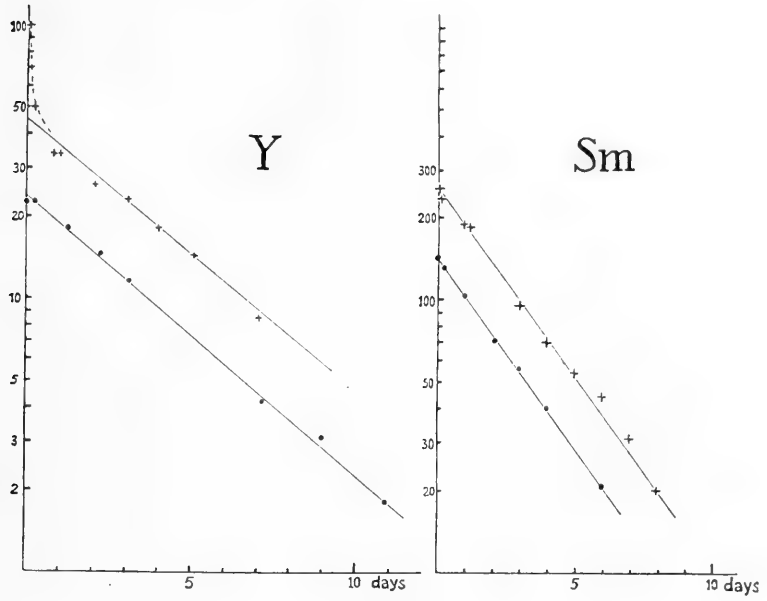

Fig. 1
a) Decay Curve of a Pure and an Impure Yttrium Preparation.
b) Samarium Decay Curves (the two days' period only; the weak period of 40 min is not visible).

cally and their separation is attended with very great difficulties. Figure 1 shows the decay of a pure preparation and of one containing some dysprosium.

Since yttrium has only one stable isotope, ^{89}Y, the artificial radioactivity obtained from it is presumably due to the formation $^{90}_{39}Y$. We find the intensity of the yttrium activity to be 0.005 times as large as that of dysprosium, both preparations having been activated until saturation was obtained in a paraffin block of $30 \times 30 \times 25$ cm edge; the neutron source was placed on the top of the preparation which was covered by a thin shield of paper. The β-rays emitted by yttrium are absorbed to half of their initial value by 0.6 mm Al.

Lanthanum

Marsh and Sugden (4) find 1.9 days as the half-life of lanthanum and for the intensity of the β-rays emitted, a value amounting to 35% of that observed for the activity of praseodymium. As we find a value of 22 for the ratio of the radiation intensities of dysprosium and praseodymium, the lanthanum activity works out at 2.0% of that of dysprosium.

As lanthanum has but one stable isotope, ^{139}La, the activity obtained is presumably due to the formation of $^{140}_{57}$La. FERMI's coefficient α, indicating the increase in activity when the bombarding neutrons are slowed down by a thick layer of paraffin or other hydrogen-containing substances, instead of being allowed to impinge directly from the beryllium source on to the substance to be activated, was found to be 12.

Cerium

No activity was observed after bombardment of cerium for several days with a neutron source of few hundred millicurie.

Praseodymium

AMALDI, FERMI, and others (1) found the artificial radioactivity of praseodymium to decay with a 19 h. period, the same value being found later by other experimenters (4), (5). Although only one stable isotope of praseodymium is known. ^{141}Pr, the above-mentioned investigators found a second period of decay (5 min) which in contrast to the first period is not hydrogen-sensitive.

Neodymium

FERMI and his collaborators (1) found that activated neodymium decays with a period of 1 h. ; we find (2) that this activity is 2500 times as small as that of dysprosium. MARSH and SUGDEN (4) found no activity, while according to MCLENNAN and RANN (5) the half-life is 35 min. Neodymium has the stable isotopes 142, 143, 144, 145, 146 and 148, and the activity observed is presumably due to the formation and decay of $^{149}_{60}$Nd.

Samarium

The artificial radioactivity of samarium decays, as was found by FERMI (1), and later by us, with a period of 40 min. We find (2) the intensity of the activity to be 0.6% of that of dysprosium. Samarium has furthermore, as was first noticed by MARSH and SUGDEN (4), a much longer period as well. We determined the period of this isotope to be 2 d., as can be seen from Fig. 1 and found its intensity to be $\frac{1}{50}$ of that of dysprosium, i. e. 2.0 on our relative scale. Samarium has the stable isotopes 144, 147, 148, 149, 150, 152, and 154, and it is not possible to determine the mass number of the active samarium isotopes with certainty.

A very intense activity was obtained by SUGDEN (3) on bombarding europium with slow neutrons. It decayed with a period of 9.2 h. The intensity of the europium radiation was found by us (2) to be 80% of the dysprosium radiation emitted by the same amount of dysprosium, both preparations being activated until saturation was reached. Care was taken, too, that the neutron beam was weakened only to a small extent by the activation process, i.e. very thin layers were activated. Europium has two stable isotopes 151 and 153 and the activity is possibly due to the formation of ^{152}Eu. The europium : dysprosium activity ratio is found to be smaller for thick layers than for thin layers.

The value 40 was found for the hydrogen-effect, α. The half-value thickness (2) of the β-rays emitted is 0.02 cm Al, and it was concluded from absorption measurements that energies up to $2.0 \cdot 10^6$ eV occur.* In addition, γ-rays have been detected which are little absorbed by 4 mm. lead.

Gadolinium

FERMI and others (1) found gadolinium to decay with a period of 8 h. after neutron bombardment. MCLENNAN and RANN (5) found a half-life of 6.4 h. and twice the intensity found for neodymium. The combination of the last mentioned figure with our intensity data leads to an intensity value which is 250 times as small as that observed for dysprosium. MARSH and SUGDEN (4) could not find any activity.

Terbium

The activity of terbium (3) decays with a period of 3.9 h. As this element has only one stable isotope, $^{159}_{65}$Tb, the activity observed is presumably due to the formation and decay of $^{160}_{65}$Tb. The intensity of the radiation (2) observed is 2.5 per cent of that of dysprosium.

Dysprosium

The activity of dysprosium (2), (4) decays with a period of 2.5 h. and is the strongest yet observed in the domain of artificial radioactivity. We have therefore chosen it (2) as a standard of comparison for the

* R. NAIDU and R. E. SIDUY (*Proc. Roy. Soc.* A **48,** 332, 36) by using a cloud chamber determined recently the energies of the β-ray spectra and found that the maximum energy lies at $1.3 \cdot 10^6$ eV, while the upper limit of the spectrum is $2.6 \cdot 10^6$ eV.

activities of the rare earth elements : we denote the intensity of dysprosium arbitrarily by 100. It is of interest to remark that the 2.3 min activity of silver, which is considered a very strong activity, is 12 times as weak as the activity of an equal amount of dysprosium. The hydrogen effect (α) was found to be 100, the half-value thickness of the β-rays emitted was 0.025 cm Al ; and the upper limit of the continuous β-spectrum concluded from absorption measurements with aluminium has an energy of $1.4 \cdot 10^6$ eV (2).* Dysprosium is one of the commoner rare earth elements of the yttria group and as it is very strongly active, activated samples of rare earth elements denoted as "erbia", "holmia", "yttria", etc. often decay with the period of dysprosium.

Holmium

We found (2) the activity of holmium to decay with a period of 35 h., while E. Rona (6) recently found the value of 33 h. The half-life of 2.6 h. measured by Marsh and Sugden (4) and later by McLennan and Rann (5) is presumably due to the presence of dysprosium in their preparations ; some of our impure preparations, too, showed an initial decay with the period of dysprosium. The samples of holmia investigated were given us by the late Baron Auer. Holmium has one stable isotope, 165 ; the activity observed is therefore presumably due to the decay of $^{166}_{67}$Ho, the intensity of the activity observed being 20 per cent of that of dysprosium. The hydrogen-effect (α) is much smaller (2) than that of dysprosium ; the half value thickness is 0.04 kgm/cm^2 Al ; and the upper limit of the β-ray spectrum has an energy of $1.6 \cdot 10^6$ eV.

Erbium

Erbium has a very weak activity of similar intensity to the 40 min samarium radiation, decaying with a 7 min period according to Marsh and Sugden (4), and with a 4.5 min according to McLennan and Rann (5). A second period (2) was found by us to be 12 h. ; the period of 2.5 h. ascertained by Sugden (3) using a commercial preparation is presumably due to the presence of dysprosium, and that found by Marsh and Sugden (4), 1.6 d., to the presence of holmium in the sample investigated. Recently Rona (6) has given the value of 13 h. for the longer period. The intensity (2) of the longer period of erbium is 0.35 per cent of that of dysprosium, and the half- value thickness of the β-rays emitted is 0.03 cm Al.

* R. Naidu and R. E. Siday, *loc. cit.* found that the maximum energy lies at $0.75 \cdot 10^6$ eV, while $1.8 \cdot 10^6$ eV is the upper limit.

This element shows an activity having a long- life as first stated by Rona (6) who finds that the β-rays emitted are half absorbed by 0.015 cm Al. E. Neuninger and E. Rona* found recently a period of 4

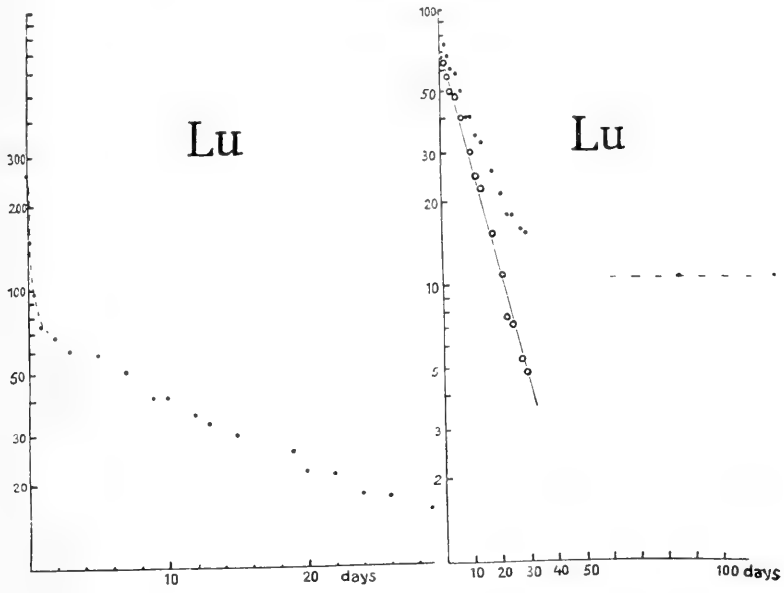

Fig. 2. Lutecium Decay Curve.
a) showing the short period and the beginning of the 7 days' period.
b) showing the measured points for the 7 days' period and the curve obtained after subtracting the residual activity.

($\pm$ ½) months. After bombarding 100 mgm TmO_2, kindly lent us by Prof. Jantsch, with about 100 mc for 23 days we obtained 60 counts per minute, while 100 mgm of dysprosium activated to saturation with the same source gave about 4000 counts per minute. The activity of this preparation decayed with a period of about 3.5 months; a thulium preparation activated to saturation would therefore exhibit an activity about $^1/_{10}$ of that of dysprosium.

Ytterbium

The activity of ytterbium (2), (3) decays with a period of 3.5 h. As ytterbium has the isotopes 171, 172, 173, 174, and 176, it cannot be decided whether the activity is due to the formation and decay of $^{175}_{70}Yb$

* E. Neuninger and E. Rona, *Wien. Anz.* **73,** 159 (1936).

or of $^{177}_{70}$Yb. The ytterbium radiation (2) is somewhat weaker than that of erbium, and amounts to 0.3 per cent of that of dysprosium. The half-value thickness of the β-rays emitted is 0.04 cm Al.

Lutecium

Lutecium (cassiopeium) exhibits an activity of fairly long life (2), namely one decaying with a period of 6—7 d., and having an intensity of 1.4 per cent of that of dysprosium; there is a second activity of somewhat less intensity decaying with a period (2) of 4 h.; as lutecium (cassiopeium) and ytterbium are very closely related elements, and lutecium being usually contaminated with ytterbium, we considered it possible that the 4 h. period observed might be due to the presence of ytterbium in the sample investigated. A very pure lutecium (cassiopeium) preparation, however, prepared by AUER and kindly lent us by Prof. HÖNIGSCHMID, also showed the 4 h. period. Furthermore the intensity of this radiation was stronger than that emitted by a pure ytterbium preparation activated by a neutron source of the same strength. So we must conclude that both the periods observed are due to lutecium. The long period of decay has not been observed by any experimenter besides us, presumably because the times of exposure have been too short. For the shorter period MCLENNAN and RANN (5) give a value of 3.6 h. and RONA (6) 4—5 h. The decay of the lutecium preparation lent us by Prof. HÖNIGSCHMID is seen in Fig. 2, the time of exposure being 2.8 days. In comparing the intensities of the long and the short periods the former must be divided by 0.267 which value follows from a consideration of the relation $J_\infty = J_t\ (1-e^{-\lambda t})$, where J_∞ is the saturation value of the activity. J_t the value obtained after t days, and λ the decay constant (= half-life/ln 2). As can be seen from the Fig. 2b a third long period is present in the activated lutecium which is possibly due to the presence of small amounts of thulium.

ABSORPTION OF SLOW NEUTRONS BY RARE EARTH ELEMENTS

Determination of the period of decay from absorption data

When faced with the problem of determining the period of very slowly decaying radioactive isotopes having half-lives of several months or years, decay measurements become very tedious. In such a case we can obtain information about the decay constant required by comparing the absorption of slow neutrons in the rare earth element in question with that in another rare earth element of known period. A knowledge of this ratio and of the activities obtained for both elements after a known

time of exposure allows us to calculate the unknown period of decay provided we can assume that all the neutrons absorbed are captured by the nuclei of the absorbing element and that mainly thermal neutrons are involved in both cases. In the oxides investigated, only the nuclei of the rare earth element absorb, for oxygen nuclei capture only a small number of neutrons. Let us consider, for example, the case of scandium. Denote by R_1 the observed absorption ratio for equal numbers of scandium and dysprosium atoms, and by R_2 the ratio of the activities obtained after an exposure of N days; then the half-life of scandium is $\frac{N \cdot R_1 \cdot 0.69}{R_2}$ days. We compared the activity of 66 mgm of scandium and 100 mgm of dysprosium and found after an activation of 24 days an activity ratio of 0.92×10^{-2}. During this activation time, full saturation of the dysprosium activity was obtained, while the scandium was far from being saturated. For equal numbers of scandium and dysprosium atoms we found an intensity ratio of 0.40×10^{-2}.

To compare the absorbing powers of scandium and dysprosium we inserted in the path of the neutron beam, which had been slowed down in the usual way by a block of paraffin, first, a layer of scandia (590 mgm/cm² Sc) and then a layer of dysprosia (340 mgm/cm² Dy) and measured the activation of a rhodium foil in the absence and then in the presence of the absorbing layer. The amounts of the absorbing material necessary to reduce the activity of rhodium in each case to 90% of its initial value were calculated to be 300 mgm/cm² Sc and 43 mgm/cm² Dy. A more satisfactory way to proceed in comparing the absorbing powers would have been to have used a dysprosium indicator to measure the absorption in dysprosium and a scandium indicator to measure the absorption in scandium, but the small activation of scandium after a few days' exposure to neutrons rendered this infeasible. We have, however, applied the last mentioned method to compare the absorption of neutrons in dysprosium, europium, and holmium, as discussed in the next section. The comparison of the absorbing powers of equal numbers of atoms of dysprosium and scandium led to the result that the former absorbed 25 times as strongly as the latter. It follows from this result and from the comparison of the activities of the two elements, that the half-life of $^{46}_{21}$Sc is about two months. A similar value was obtained by decay measurements.

Strongly absorbing rare earth isotopes forming stable products

The unusually strong activities of some rare-earth nuclei are to be ascribed to the existence of strong nuclear resonance levels in the nuclei in question, these levels corresponding to energies of slow neutrons abundant in the neutron beam passing through them, and also to the fact

TABLE 1. — ABSORPTION OF SLOW NEUTRONS IN RARE EARTH ELEMENTS
(Amount necessary to reduce the activity of the indicator by ten per cent)

Element	Indicator	mgm/cm²
Europium	Europium	13
Dysprosium	Dysprosium	40
Holmium	Holmium	120

that the isotope formed by the capture process is not a stable one already known but an active one hitherto unknown. It is a matter of experience that a mass number cannot be occupied both by a stable and an active isotope of the same element, so that should the mass number 166 be occupied by a stable dysprosium isotope the high capturing power for slow neutrons shown by $^{165}_{65}Dy$ would not lead to an active but to a stable dysprosium isotope. The appearance of a strong activity shows that at least one isotope of this element captures neutrons strongly, but high absorption does not necessarily imply strong activity because nuclei yielding stable isotopes can also be very strong absorbers of neutrons. To obtain information about the existence of strongly capturing rare-earth nuclei not leading to the formation of radioactive products, we compared the activities of dysprosium, europium, and holmium with their absorbing powers for the same neutron beam as was used to activate them. The results of these measurements, in which the absorbing element itself was used as indicator, are seen in Table 2.

TABLE 2. — ABSORPTION OF SLOW NEUTRONS IN RARE EARTH ELEMENTS
(Amount necessary to reduce the activity of the indicator by ten per cent)

Element	Indicator	mgm/cm²
Europium	Rhodium	16
Dysprosium	Rhodium	43
Holmium	Rhodium	160
Gadolinium	Rhodium	2
Samarium	Rhodium	12
Yttrium	Rhodium	500
Scandium	Rhodium	300
Cadmium	Rhodium	18

While the activity of europium is slightly smaller than that of dysprosium its absorbing power is more than twice as big ; europium thus absorbs slow neutrons to an appreciably larger extent than is to be expected from the activity of the radioactive europium isotope formed. To explain this discrepancy we have to assume that besides the

9 h. period a second period, long and therefore not observed, is present.

Samarium also shows an absorption stronger than is to be expected from the activity of the known radiactive samarium isotope. Of the numerous isotopes of samarium not leading to the formation of active isotopes at least one must therefore have a strong resonance level for slow neutrons. In view of the fairly weak activity of samarium the absorption measurements could not be carried out by using a samarium indicator, so rhodium was used for that purpose. The results of these measurements and also of absorption measurements with other rare earths using rhodium as indicator are shown in table 3.

Table 3. — Percentage of Initial Intensity of the Neutron Beam Present after the Passage of a "Thick" Layer

Element	mgm/cm²	Intensities
Samarium	580	28%
Gadolinium	120	33%
Dysprosium	610	40%
Cadmium	390	43%

It is well-known that the activity obtained by the action of slow neutrons is not a trustworthy measure of the intensity of the neutron beam, because the neutron absorbing powers of different elements are very specific and depend very much on the neutron velocities. The ambiguity arising from this fact can, however, be avoided by using the same element as indicator and absorber in absorption experiments. Should that not be feasible, as would happen if, for example, the absorbing substance did not show any or had only a very slight activity — this is the case with gadolinium — it is advisable to adopt the following procedure. The maximum absorption obtained in a thick layer of gadolinium is measured using, say, rhodium as indicator ; then the thick layer is replaced by a few milligrams of material and the absorbing power measured again. The first mentioned measurement gives the result that no more than 67% absorption can be obtained for the neutron beam in question through a thick layer of gadolinium, while the last mentioned measurement shows that 2 mgm of gadolinium are necessary to reduce the intensity of the neutron beam by 10%. To arrive at a figure giving the amount of gadolinium necessary to reduce the intensity of neutrons of such velocities as are actually absorbed in gadolinium we must multiply 2 mgm by 0.67 and thus obtain a value of 1.3 mgm. The corresponding figures for a few elements are given in Table 4 and 5. Of all the rare earth elements gadolinium —as can be seen from the table — has the

Table 4. — Absorption of Slow Neutrons in Rare Earth Elements (Amount necessary to reduce the activity of the indicator by ten per cent of that observed after passage of the neutrons through a "thick" layer)

Element	Indicator	mgm/cm²
Samarium	Rhodium	10
Gadolinium	Rhodium	1.6
Dysprosium	Rhodium	30
Cadmium	Rhodium	12

highest absorbing power ; it is indeed, as has already been shown by Dunning, Pegram, Fink, and Mitchell (8), the strongest known absorber of slow neutrons. In view of the very strong absorbing power of gadolinium great care must be taken in interpreting the results of absorption measurements on rare earth preparations which might contain traces of gadolinium. The presence of less than $^1/_2$ per cent of gadolinium in erbium, for example, would suffice to explain the whole absorption shown by erbium. As europium is often contaminated with gadolinium we used various preparations of europium to compare the absorption in europium and dysprosium. One of the preparations was kindly given us by Prof. Prandtl and was entirely free of gadolinium ; it gave a value only slightly lower than the other specimens investigated.

The high values found by different observers for the absorbing power of yttrium are clearly due to the presence of impurities in the preparations used. According to Amaldi and his collegues (1) the absorbing power of yttrium is 70 per cent of that of cadmium, and Dunning, Pegram, Fink, and Mitchell (8) give 39 per cent ; whereas using very pure preparations as described on page 48 we find that yttrium is a very

Table 5. — The Relative Activities of the Rare Earth Elements

Element Bombarded	Relative Intensity	Element Bombarded	Relative Intensity
Yttrium	0.5	Terbium	2.5
Lanthanum	2	Dysprosium ...	100
Cerium	—	Holmium	20
Praeseodymium .	4.5	Erbium	0.35
Neodymium	0.04	Thulium	12
Samarium	0.6	Ytterbium	0.25
Europium	80	Lutecium	1.4 ; 1
Gadolinium	very low		

poor absorber, its absorbing power being only 4 per cent of that of cadmium and 0.3 per cent of that of gadolinium, if the absorption of equal numbers of atoms of the different elements are compared. In Table 6 are given the relative intensities of the activities produced in the rare earth elements by neutrons that have been slowed down by large amounts of paraffin wax. We are still investigating the intensities obtained under the action of fast and semi-fast neutrons and the possible existence of resonance levels.

Comparison between the effect of neutrons on rare earth elements and other elements

As is shown in this paper numerous radioactive isotopes of the elements of the rare earth group are formed under the action of neutrons, a result which was to be expected from the known existence of a large number of stable isotopes of these elements. Thus the reactions of neutrons with the rare earth elements show the same typical features as their reactions with elements of lower and higher atomic number. The most remarkable feature is perhaps the comparatively frequent occurrence of pronounced resonance phenomena, which phenomena are much commoner among the rare earth elements than in any other part of the periodic system. This fact may be considered as a simple consequence of BOHR's theoretical considerations on neutron capture, since it would be expected that the distribution of resonance levels would be an especially close one in this region. In fact the product of the number of nuclear particles multiplied by the binding energy of a neutron in the nucleus reaches a maximum in the domain of the rare earth nuclei on account of the circumstance that the binding energy for higher particle numbers decreases considerably until — for the natural radioactive bodies — it has fallen to about half its maximum value. The more frequent occurrence of resonance capture in processes leading to the formation of stable isotopes, than in those giving radioactive isotopes is also in conformity with general experience and is easily explained by the theoretical considerations mentioned above since the distribution of levels will be much closer in the former case on account of the fact that the binding energy is considerably larger in processes of this kind than in those leading to the production of unstable isotopes.

The use of neutrons in analytical chemistry

The usual chemical methods of analysis fail, as is well-known, for most of the rare earth elements and have to be replaced by spectroscopic, X-ray, or magnetic methods. These methods can now be supplemen-

ted by the application of neutrons to analytical problems by making use both of the artificial radioactivity and of the great absorbing power of some of the rare earth elements for slow neutrons.

Qualitative analysis with the aid of artificial radioactivity is based on the determination of periods of decay. All rare earth elements have half-lives varying from a few minutes to a few month, so they can all be measured conveniently. The period of decay of 2.5 h., for example, is completely characteristic of dysprosium and is an unambiguous indication of its presence in the sample investigated; as little as 0.1 mg can be determined without difficulty. We used the method of artificial radioactivity to determine the dysprosium content of yttrium preparations. The procedure was the following: we mixed 0.1%, 1% etc. of dysprosium with neodymium oxide, the latter being chosen because it is one of the cheapest rare earth elements, having a low neutron absorbing power as has yttrium, and determined the intensity obtained. The yttrium sample to be investigated was then activated under exactly the same conditions, and a comparison of the dysprosium activities obtained gave 1% as the dysprosium content of the yttrium sample.

Another very beautiful analytical method is based on the very different absorbing powers of the different rare earth elements. A sample, 5 mgm of which spread over 1 cm^2 absorbed a quarter of the slow neutrons falling on it, could be identified at once as gadolinium, no other element having so high an absorbing power.

Unlike the method of artificial radioactivity, the absorption method is limited in its application by the fact that the absorption measure is the sum of the absorptions of the different elements present in the sample. This limitation is, however, largely due to the fact that our knowledge of the absorption of neutrons and still more our devices for producing neutrons of different energies are only in an embryonic state. The absorbing powers of different nuclei depend to a high degree on the energy of the neutrons in question and the future development of our knowledge of neutron absorption will presumably make it possible to apply absorption methods of neutron analysis of great simplicity and reliability. This method of analysis, as also that based on periods of decay, gives a direct means of identification of the nuclei involved; this distinguishes them from all other analytical methods, chemical, spectroscopic, X-ray, and magnetic, which are based on the investigation of the electronic properties of the atom in question.

Effect of neutrons on minerals containing rare earth elements

Many of the rare earth minerals, because they are products of residual magmatic crystallisation, contain rare earth elements, thorium, and uranium, along with beryllium and other light elements. The last

mentioned element is far the most effective neutron source under bombardment with α-particles or with the γ-rays emitted by uranium, thorium, and their disintegration products ; the nuclei of other elements, such as lithium, boron, magnesium, aluminium etc. are much less effective.* In minerals containing large amounts of strongly capturing rare earth elements, the neutrons produced in the mineral or in its surroundings are absorbed to a large extent in the element in question. The mineral gadolinite, for example, contains about 50% of rare earths, of which according to GOLDSCHMIDT and THOMASSEN** up to about 15% is gadolina ; this mineral often contains, too, other light elements including considerable amounts of beryllium, about 0.3% of thorium, and some uranium as well. 1 gm of thorium and its disintegration products produces up to 10^8 neutrons per year or in all 10^{16} neutrons since the formation of the minerals. If these neutrons are all absorbed in 1 kgm of the mineral in question and are absorbed primarily by the gadolinium content, 10^{16} gadolinium atoms will be formed having an atomic weight one unit higher than before the absorption. As 1 kgm gadolinite contains about 10^{22} of gadolinium atoms the equivalent weight of gadolinium will increase during that long span of time by but one unit in the fourth decimal place.

While this result is only a very rough estimate it suffices to demonstrate that some of the rare earth elements which primarily form higher stable isotopes by capturing neutrons, increase in equivalent weight as time proceeds. Dysprosium on the other hand when decaying forms holmium, holmium forms erbium etc.; the process in such cases leads to an increase in the amounts of rare earths of higher atomic number and to a corresponding decrease in the amounts of those of lower atomic number. Such behaviour is not confined to the rare earth elements ; during their presence in the earth's crust many elements heavier than zinc will undergo increases, though small ones, of their equivalent weights or of their abundance relative to the lighter elements. The first named behaviour is shown primarily by even and the last named by odd elements, because elements having an odd atomic number have always a few isotopes only so that the consecutive mass numbers are not filled by stable isotopes and the formation of radioactive isotopes through neutron addition is possible. In the case of several even elements like cadmium, tin, gadolinium, osmium, mercury, lead etc., a long series of consecutive mass numbers are filled by stable isotopes so that the capture of neutrons

* We compared the activities obtained when dysprosia was bombarded with neutrons of a beryllium-radon and a magnesium-radon source in the presence of large amounts of paraffin wax and the figures obtained were as 100 : 1.

** V. M. GOLDSCHMIDT and L. THOMASSEN, *Oslo Vidskap Selskapets Skrifter* I, Nr. **5**, S. 44 (1924).

leads chiefly to the formation of higher stable isotopes. It is therefore even elements that undergo an increase of their equivalent weights with time while the relative abundance of the elements of odd atomic number shifts towards the heavier elements.

Below zinc, conditions are very different: the result of neutron action in minerals leads here often to the formation of elements of lower atomic number and only to a smaller extent to the formation of heavier isotopes or heavier elements. For example, bombardment of aluminium leads to the formation of a magnesium isotope and to a sodium isotope; the branching ratio between these two processes depends greatly on the energy of the neutrons.

Summary

The artificial radioactivity of the rare earth elements including scandium and yttrium was investigated. The periods of decay of numerous radioactive isotopes produced lie between 5 min. and a few month. The biggest and smallest saturation intensities of the radiation emitted by these isotopes are in the ratio 10,000 : 1. The half-value thickness in aluminium of the β-radiation emitted was measured in several cases, and, in some cases, the maximum energy of the continuous β-ray spectrum and Fermi's constant a as well.

The absorption of slow neutrons in rare earth elements was measured with a view to discovering the presence of strongly absorbing nuclei not giving rise to active isotopes.

The application of artificial radioactivity to analytical chemistry is discussed.

It is shown that the combination weight of the rare earth elements occurring in minerals in which a continual production of neutrons takes place has undergone a slight change during geological time.

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5. ARTIFICIAL ACTIVITY OF HAFNIUM AND SOME OTHER ELEMENTS

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ARTIFICIAL RADIOACTIVITY OF HAFNIUM

Some time ago we found that under the action of neutron bombardment a radioactive isotope of hafnium is produced, the activity decaying with a period of a few months (4). To determine the period of decay more exactly, we activated 280 mgm of hafnium oxide prepared by one of us (3) by placing it in a paraffin block together with radium-beryllium sources, containing 600 mgm of radium element as sulphate and twenty times as much metallic beryllium powder. After irradiation for three months the hafnium oxide was removed from the paraffin block and put into an aluminium dish having a surface of 1.2 cm^2 and a height of 2 mm. The dish was placed directly below the aluminium window of our counter, the window having a thickness of about 20 μ. We followed the decay of the hafnium preparation for 200 days by comparing its activity with that of an uranium standard. The decay curve obtained is seen in Fig. 1 and Table 1.

From the latter we can conclude that the half life of hafnium is 55 ± 10 days (standard mean square deviation). Our initial activity was 20 counts per min, the natural effect being about 4 counts per min. We followed the decay curve until we had a net activity of 1 counts per min. From the fact that, in spite of the long activation, such a modest

Table 1. — Decay-measurement of Hafnium

Date	Nr. of Days	Counts/min
17. VIII. 36.....	0	16.1
18. VIII. 36.....	1	15.3
24. VIII. 36.....	7	13.7
12. IX. 36.....	25	12.0
29. X. 36.....	73	7.8
7. I. 37.....	143	2.3
22. I. 37.....	158	1.8
25. II. 37.....	192	1.0

activity was obtained we can conclude that hafnium does not belong to the elements showing a strong artificial radioactivity. This is partly due to the fact that the capture of neutrons by most of the hafnium isotopes leads, as explained later, to the formation of a heavier stable

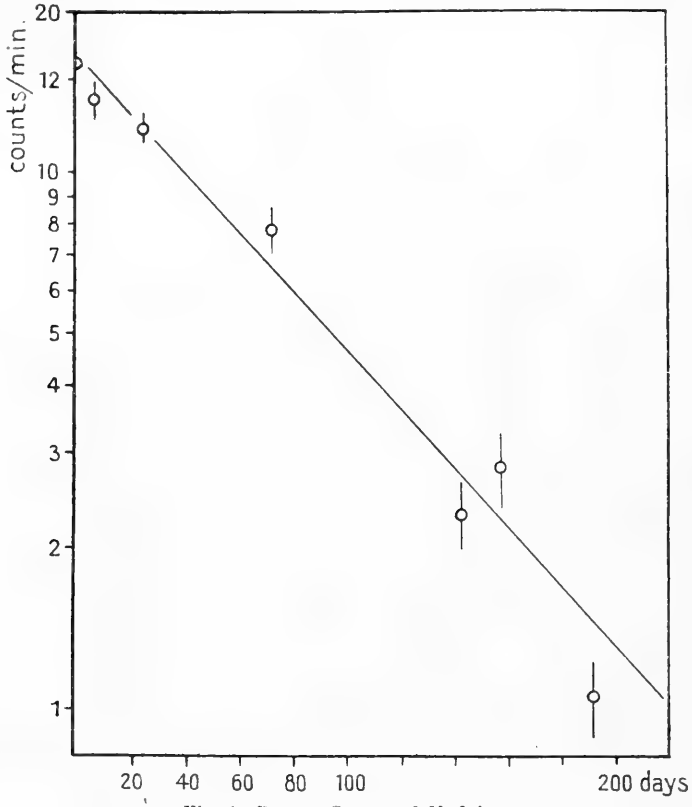

Fig. 1. Decay Curve of Hafnium.

isotope ; as stable isotopes 176, 177, 178, 179, and 180 are known and only the absorption of neutrons by the last mentioned isotope can lead to the formation of an active product. The relative abundance of the isotopes in the naturally occurring element hafnium, as determined by Aston, is seen from Table 2.

Table 2. — Relative Abundance of the Hafnium Isotopes

Mass number	Abundance
176	5%
177	19%
178	28%
179	18%
180	30%

We measured also the absorption in aluminium of the β-rays emitted by hafnium. The values obtained are seen from Table 3.

TABLE 3. — ABSORPTION IN ALUMINIUM OF THE β-RAYS EMITTED BY HAFNIUM

Thickness of the Al-foil	Counts/min.
0	15.8
11 mgm/cm^2	10.2
16.5 mgm/cm^2	7.4

(half value thickness : 16 ± 1 mgm/cm^2).

From the figures in Table 3 follows that an aluminium layer of 16 mgm per cm^2 reduces the intensity of the β-rays emitted by a hafnium oxide layer of 230 mgm/cm^2 to one half of its initial value. The comparison of the absorbing power of aluminium for the β-rays of hafnium and scandium, decaying with periods of 55 and 90 days respectively, shows no great difference ; the ratio of the two half-value thicknesses being 1.2. The softness of the hafnium radiation is partly responsible for the low activities obtained after long exposure of hafnium oxide with radium-beryllium sources of a few hundred millicurie, the β-radiation emitted being absorbed to an appreciable extent in the hafnium oxide sample itself. In the case of hafnium, as already mentioned, every place between the mass numbers 176 and 180 is occupied by a known stable isotope ; the formation of the active hafnium isotope is presumably due to the process

$$^{180}_{72}\mathrm{Hf} + ^{1}_{0}\mathrm{n} = ^{181}_{72}\mathrm{Hf}.$$

On emitting β-rays according to the equation

$$^{181}_{72}\mathrm{Hf} = ^{181}_{73}\mathrm{Ta} + \beta$$

the active hafnium isotope becomes the only stable isotope of tantalum known. Hafnium is thus partly converted into tantalum under the action of neutron bombardment, while, as shown by us previously, hafnium is formed under the action of neutrons on lutecium. It is quite possible that, under bombardment with a powerful stream of deuterium or of neutrons, further decay periods of hafnium will be discovered.

THE EFFECT OF NEUTRON BOMBARDMENT ON SCANDIUM

A few years ago we embarked on the investigation of the effect of neutron bombardment on scandium, (4), (5), (6), chiefly in the hope of being able to prepare an artificial radioactive isotope of potassium and to ob-

tain some information on the then not entirely elucidated nature of the natural radioactivity of potassium. We bombarded a few grams of very pure scandium oxide prepared by Prof. STERBA-BÖHM and used by Prof. HÖNIGSCHMIDT in his work on the atomic weight of scandium. After neutron bombardment the scandium oxide was dissolved in dilute hydrochloric acid and 100—150 mgm of sodium chloride as a carrier of ^{42}K and the same amount of calcium oxide was added. The filtrate obtained after precipitation with carbonate-free ammonia was treated with oxalic

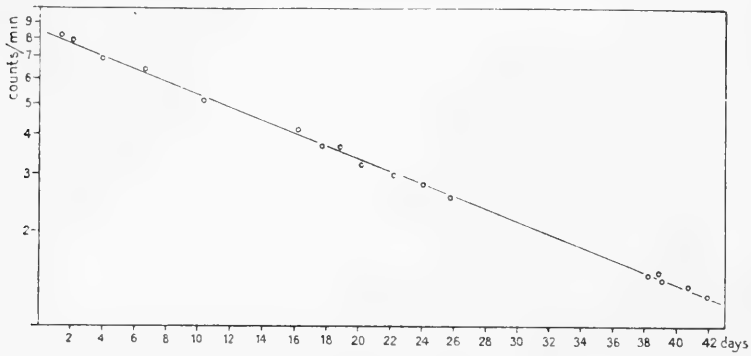

Fig. 2. Decay Curve of the Potassium Precipitate.

acid and the calcium oxalate formed was removed. The sodium chloride which had been added to the solution of the scandium chloride compound was recovered after the removal of the ammonium chloride content of the last filtrate by evaporation. The activities of the three fractions, namely scandium oxide, sodium chloride, and calcium oxalate, were then determined. Only the two first preparations mentioned were found to be active. The activity of the scandium oxide decayed very slowly while the various sodium chloride fractions obtained in different experiments lost half of their slight activity within 10 and 18 hours. We had just finished the experiment mentioned when a note was published by FERMI and his collaborators (1) concerning the action of neutrons on potassium. They found that potassium captured neutrons by giving birth to a potassium isotope decaying with a half-life of 16 hours. The values found by us for the period of the slight activity of different potassium preparations obtained from irradiated scandium showed a half-life between 10 and 18 hours; we thought it justifiable, therefore, to identify the element found by us with that found by FERMI and his collaborators. The initial activities measured amounted usually to about 10 counts/min. In one case, through the courtesy of the Medical Radium Station and Dr. J. C. JACOBSEN, we obtained an unusually strong neutron source containing 600 millicuries radium-emanation. The decay curve obtained for potassium 42 in this experiment is seen from Fig. 2.

The activity found by us in the filtrate of scandium precipitate could only be that of ^{42}K, as the presence of active impurities was excluded by the fact that the above mentioned very pure scandium sample was used. The possibility that we measured the half-life of radioactive sodium of 15 hours can be excluded with certainty not only for the reason mentioned above but also for the following reasons: Sodium, $^{24}_{11}Na$, can be prepared either from ^{23}Na by simple neutron capture, or from magnesium if the capture is followed by emission of a proton, or from aluminium if the capture is followed by emission of an α-particle. From the first mentioned process with the neutron sources at our disposal only very weak activities can be obtained even when starting with pure sodium. To prepare measurable amounts of radio-sodium from a few grams of impure scandium oxide an appreciable amount of magnesium or aluminium would have had to be present in the preparation. 15 mgm of aluminium mixed with 150 mgm of ammonium nitrate, for example, gave after activation to saturation less than 0.5 counts/min. and the activity obtained by similar amounts of magnesium with the same sources as used when activating scandium was still smaller. The amount of radio-sodium obtained from magnesium is less, and that of sodium by neutron capture very appreciably less than that obtained from aluminium. Scandium having just one stable isotope $^{45}_{21}Sc$, only the potassium isotope ^{42}K can be produced under neutron bombardment according to the equation

$$^{45}_{21}Sc + ^{1}_{0}n = ^{42}_{19}K + ^{4}_{2}He.$$

In the case of neutron capture by potassium, on the other hand both reactions $^{39}K + n$ and $^{41}K + n$ can occur. While FERMI and his collaborators left it open which of the two last named potassium isotopes were produced, we could conclude from our experiments that the process witnessed by FERMI was $^{41}K + n = ^{42}K$, and also that the process ^{39}K very probably leads to the formation of the potassium isotope ^{40}K which is responsible for the natural radioactivity of potassium. Recently, WALKE (9), by making use of LAWRENCE's powerful cyclotron, which supplies a many thousand times stronger neutron beam as obtained from our radium-beryllium sources, was able to follow the decay of ^{42}K through ten periods and determined its half life period to be 12.4 ± 0.2 hours, i. e., a somewhat lower value than that following from the investigations of FERMI and from our Fig. 2.

Besides preparing ^{42}K according to the equation

$$^{45}_{21}Sc + ^{1}_{0}n = ^{42}_{19}K + \alpha\ .$$

we succeeded (4) also in preparing this isotope by the process

$$^{42}_{20}Ca + ^{1}_{0}n = ^{42}_{19}K + ^{1}_{1}H\ .$$

Walke (10) while reproducing Fermi's results and also ours as to the preparation of ^{42}K from scandium, was unable to reproduce our experiments in which ^{42}K was prepared from calcium. This negative result induced us to repeate our experiments, this time by bombarding with fast neutrons as much as 1 kgm of calcium carbonate. These were dissolved in a minimum amount of HCl, precipitated by a minimum amount of ammonium oxalate, which sufficed to precipitate all calcium after dissolving 100 mgm of sodium chloride as carrier. Before we finished these experiments, a second paper of Walke (12), (13), was published in which he describes succesful experiments in producing ^{42}K from calcium, thus corroborating our statement.

ACTIVITY OF SCANDIUM

After the removal of the radio-potassium produced, the scandium was still showing a weak activity which could not be removed by chemical operations and which is possibly due to a radioactive isotope of scandium. The decay of the weak activity of scandium observed for 240 days is seen from Table 4, which shows the presence of a very weak activity decaying with a period longer than a year. We could not follow up this very weak activity further but concentrated our interest on another period obtained after activating for 24 days in a paraffin block which contained emanation-beryllium sources of an average strength of 50 millicuries. The result obtained is seen from Fig. 3a ; the half- life works out to be 90 ± 5 days.

Table 4. — Activity of a Scandium Sample after Removal of Potassium

Date	Nr. of Days	Counts/min
5. III. 36.....	0	7.3 ± 0.6
21. III. 36.....	16	$6.0 + 0.4$
24. IV. 36.....	50	7.2 ± 0.4
20. V. 36.....	76	5.1 ± 0.4
5. VI. 36.....	92	$5.7 + 0.4$
1. VII. 36.....	117	$4.9 + 0.3$
13. VIII. 36.....	161	$5.3 + 0.4$
24. X. 36.....	233	$4.4 + 0.3$

In the next set of experiments we activated simultaneously three scandium preparations for 50 days with radium-beryllium sources of a strength of about 200 millicuries : one in the usual way inside the

paraffin block, the second one in a paraffin block but with the preparation surrounded by a shield of cadmium, which absorbed nearly 100% of the C-neutrons, and the third one with fast neutrons. The result of the activation of the first named sample is seen from Fig. 3b. The investigation of the second sample led to the result that in the presence of

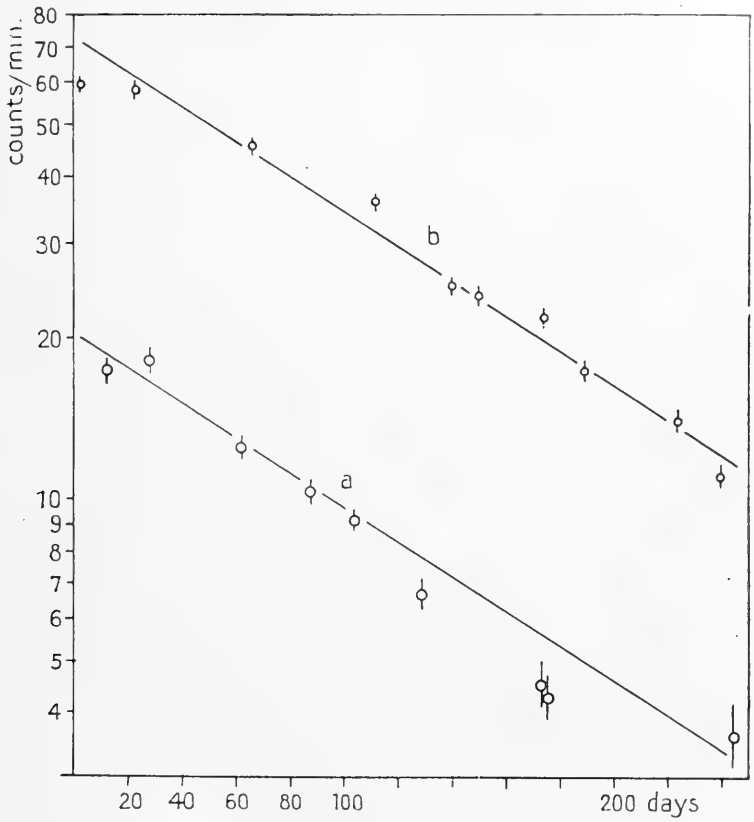

Fig. 3. Decay Curve of Scandium Irradiated in a Paraffin Block for a) 24 Days b) 50 Days.

cadmium the artificial radioactivity of scandium is reduced to 2% of the value obtained in the absence of cadmium. From Fig. 3 there follows for the half-life period of scandium the value 90 ± 5 days. Quite recently WALKE (11), by making use of LAWRENCE's powerful cyclotron, bombarded scandium with deuterons and obtained a period of 85 ± 2 days.

We want furthermore to mention an early experiment in which we bombarded scandium with fast neutrons emitted by a mixture of 600 millicuries emanation and beryllium powder ; we observed a period of decay of about 50 hours. As shown by POOL, CORK, and THORNTON (8),

and by WALKE (12), under bombardment with fast neutrons the following two reactions occur as well :

$$^{45}_{21}\mathrm{Sc} + ^{1}_{0}\mathrm{n} = ^{43}_{21}\mathrm{Sc} + 3\ ^{1}_{0}\mathrm{n}$$

$$^{54}_{21}\mathrm{Sc} + ^{1}_{0}\mathrm{n} = ^{44}_{21}\mathrm{Sc} + 2\ ^{1}_{0}\mathrm{n}$$

the scandium isotopes obtained emit positrons and have half-lives of 4 and 43 hours respectively ; it was presumably the last mentioned reaction which we observed.

Scandium 44 was also produced (13) by the action of α-particles on potassium 41 and (11) by the action of deuterons on calcium 43, while scandium 43 was produced (11) by the action of α-particles on calcium 40, and (2) by the action of deuterons on calcium 42.

WALKE was furthermore successful in producing scandium 42 under the action of α-particles on potassium 39, and of scandium 41 under the action of deuterons on calcium. The list of the known radioactive isotopes of scandium is seen from Table 5.

TABLE 5. — ACTIVE ISOTOPES OF SCANDIUM
(According to Walke [11])

Active Isotope	Particle Emitted	Half-life
^{41}Sc	positron	53 min.
^{42}Sc	positron	4.1 h
^{43}Sc	positron	4.0 h
^{44}Sc	positron	52 h
^{46}Sc	electron	85 days and possibly also a period of about 1 year.

We measured the reduction of counts when covering an active scandium preparation decaying with a period of 90 days with aluminium foils of varying thickness. The result obtained can be seen from Table 6.

TABLE 6. — ABSORPTION IN ALUMINIUM OF THE β-RAYS EMITTED BY SCANDIUM

	Thickness of the Al-foil	Counts/min
1st experiment	0	12.5
	5.5 mgm/cm^2	8.8
	11.0 mgm/cm^2	7.1
2nd experiment	0	61.8
	11.0 mgm/cm^2	35.3
	16.5 mgm/cm^2	25.8

(half- value thickness : 13 ± 1 mgm/cm^2)

In view of the softness of the β-rays emitted we used thin scandium oxide layers; about 50 mgm/cm^2. In spite of the thin layers used the soft components were absorbed in the preparation to an appreciably greater extent than were the hard ones; accordingly we have to reckon with the possibility that the radiation emitted by scandium is still softer than indicated by the figures of Table 6.

THE RADIOACTIVITY OF EUROPIUM AND ITS ANALYTICAL APPLICATION

In their fundamental research on the action of neutrons FERMI and his collaborators (1) investigated also the activity of a gadolinium preparation bombarded by neutrons and found an activity decaying with a period of 8 hours. A few years later, SUGDEN (7), investigating the radioactivity of europium, discovered a very strong activity decaying with a period of 9.2 h. and, at that time, interpreted the above mentioned period of decay of gadolinium to be due to the presence of some europium in the sample investigated. Investigations carried out by us, in which we made use of different gadolinium samples prepared by Prof. ROLLA and partly by Prof. PRANDTL and the late BARON AUER v. WELSBACH, confermed completely the conclusion arrived at by SUGDEN, and this induced us to make use of the radioactivity of europium produced under the action of neutrons to determine the amount of europium present in gadolinium preparations. Prof. ROLLA, being engaged in the preparation of large amounts of pure gadolinium compounds, sent us several samples, the europium content of which he wished ascertained. We describe in the following the analytical procedure used by us.

Thin layers of the gadolinium oxide samples to be investigated were fixed between two glass plates and placed within a paraffin block. Usually we investigated simultaneously the activation of 4 symmetrically placed preparations. It is of importance to bombard layers having the same thickness and to bombard them with neutrons in such a way that each preparation is hit by the same number of neutrons; the latter was achieved by arranging the sources in the block circularly. We used in these experiments radium-beryllium sources containing 600 mgm of radium, the neutron emission of which corresponds to that from about 400 millicuries of radium emanation; in addition a beryllium-emanation mixture containing 300 millicuries emanation was also present. After irradiating the samples for 3 days they were homoginized and each sample placed in a small aluminium dish having a surface of 1.2 cm^2 and put below the window of a Geiger-counter. The intensity of the activity of the different gadolinium samples investigated is proportional to their europium content. In order to arrive at a figure stating

the europium concentration, we added 2% of europium oxide to a pure gadolinium preparation denoted as standard sample in Table 7 and compared the activity of the latter with that of the gadolinium preparations of unknown europium content. The results are seen from Table 7.

TABLE 7. — ACTIVITY OF DIFFERENT GADOLINIUM PREPARATIONS
(The sample labelled "Standard" is the Gd_2O_3 to which 2% Eu_2O_3 was added: samples 1—4 represent progressiv stages in the purification process carried out by Prof. ROLLA)

Samples	counts/min
"Standard"	125
1	60
2	60
3	30
4	25

That, in spite of the large amount of radium and emanation used, the activities measured were not stronger is partly due to the high absorbing power of gadolinium, which reduces the density of thermal neutrons. This effect is especially marked on account of the fact that the thermal neutrons diffuse and are likely to pass through the preparation several times. The latter effect can be best estimated by comparing the activity of pure europium oxide with that obtained when this material is embedded in gadolinium oxide. We activated simultaneously 200 mgm of europium oxide and 200 mgm of gadolinium oxide containing 2% of europium. If gadolinium absorbed to the same extent as europium, the first named preparation should be 50 times more active than the last mentioned one. Actually we find the ratio to be 200 from which it follows that the presence of gadolinium in our preparations reduced the activity of europium to ¼ of the value which would have been obtained if the same amount of europium oxide had been subjected to irradiation.

Summary

The irradiation of hafnium with neutrons has been shown to produce a radioactivity with a half-life of 55 ± 7 days which may be ascribed to $^{181}_{72}Hf$. The intensity of the β-rays emitted is reduced to half of its initial value by an aluminium foil having a weight of 16 mgm/cm².

Scandium, $^{46}_{21}Sc$, was found to decay with a half-life of 90 ± 5 days. The half value thickness for the absorption in aluminium of the β-rays from this element was found to be 13 mgm/cm².

The europium content of gadolinium oxide samples prepared by Professor LUIGI ROLLA was determined by making use of the artificial radioactivity produced under the action of neutrons on the europium present in his samples.

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Comment on papers 4 and 5

Usually the radioactive indicator must be added to the element the atoms of which are to be traced. It is, however, also possible to produce the radioactive tracer *in situ* by bombarding the sample with a neutron stream or other energy-rich radiation.

In contrast to present days very few people disposed of rare-earth elements before World War II. Among those was my friend Professor Luigi Rolla, professor at the University of Florence. We used to analyse his samples by making use of the method of X-rays analysis described in the comment to papers 3 and 15. After preparing a few kilograms of gadolinium oxide he wished to find out whether or not his samples were free from Eu_2O_3, the most likely impurity present in Gd_2O_3. At that date we had no X-ray spectrograph at our disposal and in order not to disappoint Professor Rolla we tried to answer the above question by exposing 50 mgm of his sample in a paraffin block to the effect of neutrons emitted by a mixture of 600 mgm radium and beryllium and 300 Mc of radon and beryllium. Neutron sources were placed in the paraffin block to obtain slow neutrons which are strongly captured by europium producing a radioactive europium isotope. They are strongly absorbed by gadolinium as well, their absorption leading, however, to the production of stable gadolinium isotopes and not to a radioactive isotope of this element ; the latter can only be produced by more intense neutron streams than applied in our experiment. The presence of an activity in Rolla's exposed samples decaying with a half-time period of 9.2 hr indicated the presence of some europium in his preparations. To carry out a quantitative analysis we added to a known amount of pure Gd_2O_3 (obtained from the great rare-earth chemist Auer von Welsbach) known amounts of pure Eu_2O_3 (also obtained from him). The comparison of the activity of Rolla's samples with those of these standard preparations lead to the result that Rolla's purest gadolinium oxide sample contained 0.40, his least pure sample 0.96 per cent of europium oxide.

We had already previously, as described in paper 4, applied this method of activation analysis in the determination of dysprosium present in yttrium samples. The determination of europium in gadolinium is unsurpassed in its simplicity and sensitivity. Europium being the element which can be determined with the greatest sensitivity by activation analysis. We were thus fortunate to be faced with the task of applying this newly introduced method in a case which proved later to be the most favourable one. The modest neutron flux emitted by our radium-beryllium sources allowed not less than 0.01 per cent of europium to be determined. By making use of the neutron flux of the cyclotron Seaborg and Livingwood could determine 6 p. p. m. of gadolinium in iron by activation analysis and after the availability of pile-emitted neutrons of great density such small amounts of europium could be determined as 10^{-14} mgm In the determination of gadolinium we availed ourselves of the very high absorbing power of this element for slow neutrons, thus of an absorbtion method.

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6. THE PROBLEM OF THE ISOTOPIC ELEMENTS

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1. THE ISOTOPE CONCEPT

It is well known that the separation methods of analytical chemistry have failed when dealing with some radioelements: Nobody has ever succeeded in separating radium-D from lead, mesothorium from radium, or ionium from thorium, nor has it once been possible in these and numerous other cases to achieve even a slight enrichment. As more unsuccessful experiments became known, the workers in this field adopted the view that they were concerned with an inseparability of quite a different kind from that operative with, for example, the rare earths. F. Soddy[1] was the first to give clear expression to this view by designating such elements as "chemically and physically practically identical" and also to search systematically for new examples of such inseparability among the radioelements[2].

Especially striking in connexion with the inseparable elements was the fact that they frequently have considerably different atomic weights which, since the α-particle was known to be identical with the helium atom, could be calculated in many instances with certainty; for example, the end product of the uranium series, radium-G, which is generally regarded as lead, must have an atomic weight different from that of ordinary lead[3]. Confirmation of the correctness of this conclusion has been obtained from the recently performed determinations of atomic weights[4], which demonstrated that the lead from pitchblende has in fact an appreciably lower atomic weight than ordinary lead and the lead from thorium minerals.

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The question of the identity of different elements was given increased attention since this was the basis of arranging the radioelements in the periodic system[1]. K. FAJANS[2] has indicated that this idea can be carried throughout the periodic system and that the ordinary elements also are probably mixtures. FAJANS has given the tame "pleiade" to such a group of elements which occupy the same position in the periodic system; the separate members were called "isotopic" elements by SODDY. The lack of an ionium spectrum in ionium-thorium samples[3] could scarcely be explained on any assumption other than that the isotopic elements show no differences in their spectra.

The theory of isotopic elements was not readily acceptable to chemists and physicists; to the former, because ever since the formulation of the periodic system they had been accustomed to regard the atomic weight as a fundamental property of an element; to the latter, because there was no known instance in which two different elements exhibited the same spectrum and such a hypothesis seemed difficult to unify with the prevailing ideas on the origin of spectrum lines[4]. These doubts were removed and the whole concept of the nature of isotopic elements was simultaneously given considerably more weight by the ideas, developed by E. RUTHERFORD[5] and N. BOHR[6], on the constitution of the atom, and by the experiments of MOSELEY[7] on the X-ray spectra of the elements. According to the Rutherford model of the atom, the mass of the atom is associated with an extremely small volume at the positively charged centre and the number of positive charges, and not the atomic weight, is primarily responsible for the properties of the corresponding element. Since the number of electrons which occupy the volume between the nucleus and surface of the atom is given by the size of charge on the positive nucleus and all chemical and physical properties of the element depend on the number and arrangement of these electrons; gravitation and radioactivity are excepted. Instability of the nucleus results in radioactive phenomena and the fact that the nuclei of two atoms have the same charge and the same physical and chemical properties but different mass and stability (e. g. radium-D and lead) agrees very well with the Rutherford-Bohr theory.

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[6] N. BOHR, *Phil. Mag.* **26,** 1 (1913).

[7] H. MOSELEY, *Phil. Mag.* **26,** 1024 (1913).

Determination of the charge on the nucleus can be made approximately as a result of the experiments on scattering of α-particles by M. Geiger and E. Marsden, and more accurately by the recently performed study by H. Moseley on X-ray spectra. A knowledge of the wavelength of the characteristic X-radiation of an element permits calculation of the nuclear charge when certain assumptions are made; it was thus found that this charge always increases by unity on moving from one position in the periodic system to the next higher[1]. Generally this means a climb to the element with the next higher atomic weight but, in a few exceptional cases, where the chemical properties force the element with the lower atomic weight to be arranged higher in the system (e. g. cobalt and nickel), the rule stated above still applies and thus demonstrates that the number of charges, and not the atomic weight, determines the position of an element in the periodic system. Accordingly, the separate positions can be numbered by stating the nuclear charge; aluminium, for example, thus acquires the atomic number 13, gold 79, etc., and between these all the available numbers except three are already representative of known elements. E. Rutherford and C. Andrade[2] have proved directly, by determining the X-ray spectrum of radium-B, which was found to be the same as that of lead, that there are elements having different atomic weight but the same nuclear charge.

Isotopic elements differ, according to this observation, only in the structure and mass of the nucleus. The structure does not enter into the ordinary physics and chemistry but is only of importance to the radioactivity. The radioactive properties, however, were the chief means of differentiating the isotopic element and, with a few exceptions (metaneon, the different kinds of lead), even now we are only aware of the existence of such isotopic elements in those examples in which at least one of them is radioactive. Separation by utilizing the radioactive differences does not seem to be conceivable; it is otherwise with the second fundamental property of the nucleus, gravitation, which should permit both distinguishing and separating.

It is useful in these discussions to distinguish between the gravitational and electronic properties; in all applications of weighing (primarily determinations of atomic weight and of solubility[3], etc.) differences in weight of the atoms are directly of use, and diffusion in the vapour-state also depends noticeably on the mass and even permits separation; Aston[4] has thus succeeded in fractionating metaneon and neon. Centrifuging also is a process in which mass plays a part and can be applied in

[1] H. Moseley, *Phil. Mag.* **26,** 1024 (1913); *Ibid.* **27,** 705 (1914). See also A. van den Broek, *Phys. Z.* **14,** 32 (1913).

[2] E. Rutherford and C. Andrade, *Phil. Mag.* May (1914).

[3] K. Fajans, *Naturwissenschaften* **2,** 544 (1914).

[4] Aston, *British Association Report*, Birmingham (1913).

several cases for separation. On the other hand, the theory mentioned above considers the chemical properties as essentially independent of the mass, and this applies also to the spectrum and radius of the atom.

Differentiation between gravitational and electronic properties is naturally only clear-cut in limiting cases; for example, the velocity of diffusion in liquids, which is primarily governed by the radius, is not independent of the mass[1] and, according to BOHR, the same should also apply in respect of the Rydberg constant of the spectrum series[2]; a difference in atomic weight of 1 per cent affects the latter quantity by about 0.05 per cent. The characteristic vibrations of the molecules in the space lattice, and consequently the specific heats, also are probably noticeably different in isotopes[3].

2. CAN ISOTOPIC ELEMENTS REPLACE EACH OTHER CHEMICALLY?

From the above discussions it is evident that isotopic elements are certainly not truly identical; the question now is whether they can be denoted as chemically identical, i. e. whether they can replace each other in their chemical mass action. It is well known that the concentration of substances taking part in all chemical reactions is important (law of mass action of GULDBERG and WAAGE); if isotopes are chemically identical the concentration must be represented by the sum of the isotopic elements present. For example, the solubility product of barium-free radium-mesothorium chloride would be written in the form

$$[\mathrm{Ra}^{**} + \text{mesothorium}^{**}]\ [\mathrm{Cl}]^2 = K$$

Now there is a particularly clear method of testing for replaceability. In electrochemical processes a jump in potential is determined by the concentration of ions of the metal involved; now when two elements (A and B) are replaceable, the addition of ions of the element B to those of A should exercise the same effect on the potential jump as if the element A had been raised to the ionic concentration A + B. For example, the potential difference RaD metal/RaD nitrate solution should be changed to the same extent by the addition of lead nitrate solution, within the meaning of Nernst's theory of the galvanic production of current, as if the ionic concentration of RaD had been increased, and vice versa.

Instead of the electrode potential of a metal, the so-called decomposition potential, which, according to LE BLANC, is of the same magnitude

[1] G. HEVESY, *Phys. Z.* **14,** 1209 (1913).
[2] N. BOHR, *Phil. Mag.* **27,** 512 (1914).
[3] K. FAJANS, *Naturwissenschaften* **2,** 544 (1914).

and is the voltage at which the element can be deposited electrolytically, can be considered. This was the first method which we adopted to solve the above problem, namely, to determine whether the decomposition potential of an element is displaced when an isotopic element is added to it. The sensitivity of radioactive methods permits the quantitative determination of even the unweighable amounts which always deposit below the decomposition potential, and this opened up a second method of testing the problem ; we studied the variation in these amounts on adding isotopic elements. The third method depended directly on measuring the potential difference shown by a RaD peroxide electrode. More details will be discussed below concerning the method of depositing RaD peroxide which we have succeeded in preparing from radium emanation in visible amounts.

3. STUDIES ON THE REPLACEABILITY OF ISOTOPES

(a) The Decomposition Potential of Radioelements

When determining the curve of the decomposition potential it is usual to measure the current passed by the cell as a function of the electrode potential. In plotting these curves it is always postulated that the current is carried essentially by the ion whose decomposition potential is to be determined and that current can pass continuously only when the potential difference attained at the cathode is equal to that which would be registered when the metal in question is immersed in the solution. This method of determining decomposition potentials is not applicable in radio-electrochemistry since the concentration of the radioions is not sufficient to carry the current exclusively. Therefore, we studied the amounts of the radioelements deposited during a time of 24 hr, under precisely the same conditions, as a function of the cathode potential. In the first method a sudden increase in the current strength occurred at the value of the decomposition potential ; in the second method there was a sudden increase in the amount deposited ; a further difference between the two types of decomposition-potential curves consists in that the deposition in the second type can be investigated at potentials even higher than the decomposition potential whereas in the the first type the cathode potential does not rise even when the current is increased.

We have plotted in Fig. 1 a decomposition-potential curve of the second type for radium-E ; the solution was about 10^{-9}N in RaE (isotopic with bismuth).

It is evident from the curve that some RaE is deposited at any potential and that because of the sensitivity of the method this amount can be

determined quantitatively but that at —0.24 V (compared with the calomel electrode) there occurs a sudden increase in the amount deposited. If bismuth nitrate is now added to the solution until the Bi + RaE normality of 10^{-4} is reached the characteristic increase takes place at —0.14 V i.e., about 100 mV lower (see Fig. 2). According to NERNST's theory it can be expected that a change in concentration of

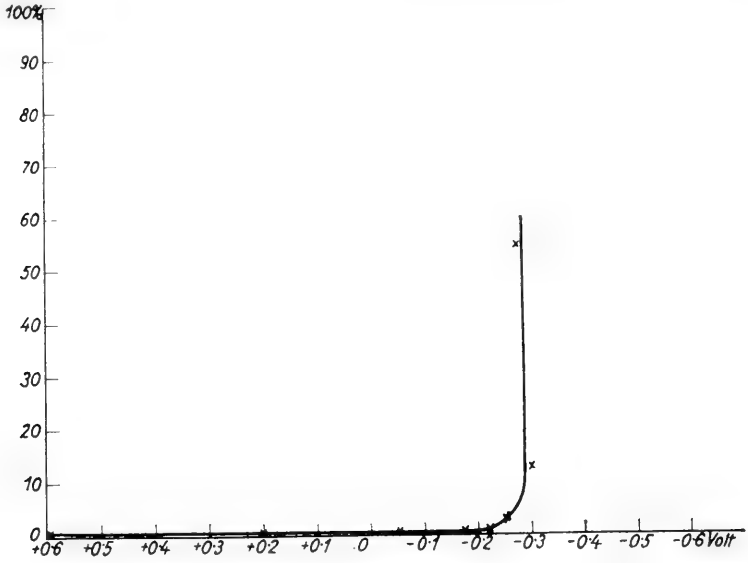

FIG. 1. Cathodic deposition of radium-E. Concentration of the solution about 10^{-9}N in RaE

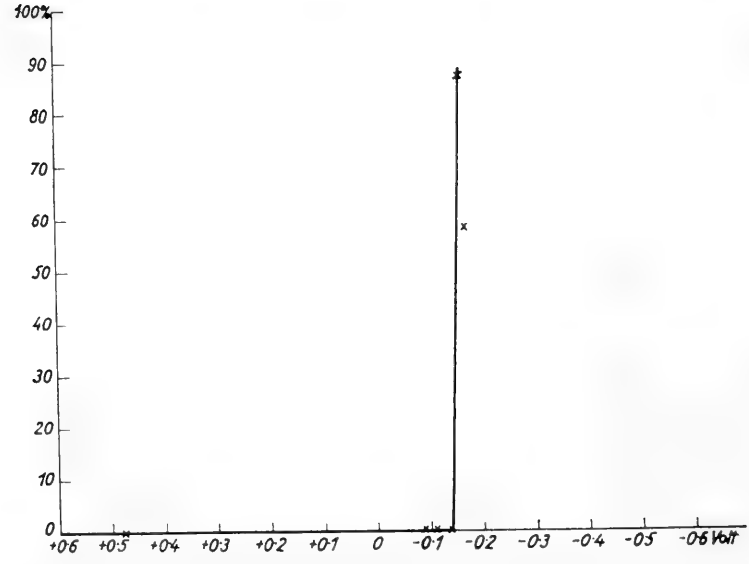

FIG. 2. Cathodic deposition of radium-E. Concentration of the solution 10^{-4}N in bismuth isotopes, Bi + RaE.

trivalent Bi by a power of ten will result in a lowering of the decomposition potential by about 18 mV ; in the present example, therefore, 90 mV would be expected. The break in the curve for pure RaE is indeed distinct but after all not so sharp as that for RaE + Bi ; this is an effect which in the first case is connected with the fact that the electrode could not be covered with a layer of RaE even if all the RaE present were deposited.

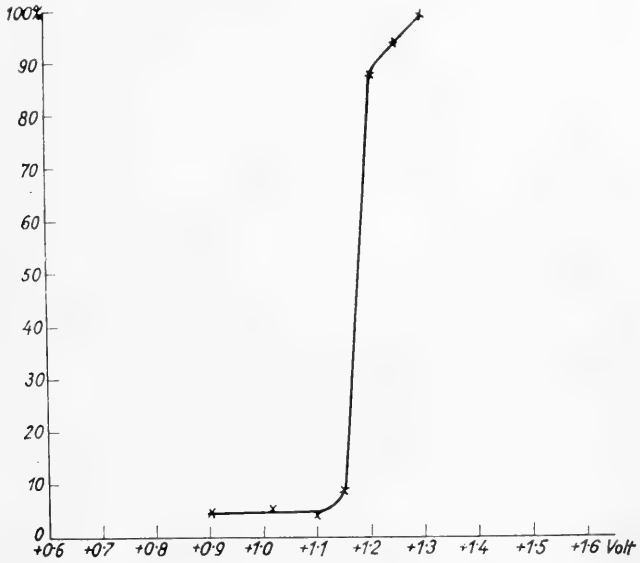

Fig. 3. Anodic deposition of thorium-B peroxide. Concentration of the solution 10^{-12}N in ThB.

The lack of sharpness becomes still more pronounced with more dilute solutions, e. g. in the case of our experiments with ThB. The solution was about 10^{-12}N in ThB. The discontinuity for peroxide deposition, which can be traced more easily than that for metallic thorium-B, occurs at + 1.13 V (see Fig. 3). Since the decomposition potential in 0.001 N lead nitrate solution saturated with PbO_2 occurs at 0.87 V, the displacement amounts to 0.26 V. From the concentration difference of nine powers of ten a difference of $9 \times 28 = 252$ mV would be expected from theory, and thus the values agree very well[1]. Individual difficulties which have been encountered in these determinations will be examined in the discussion of the experimental details.

[1] If the average value of 20 mV determined by Cumming and Abegg (*Z. Elektrochem.* **13,** 19 [1907]) is assumed as the displacement per power of ten, then the agreement is less good, yet always passable in view of the large sources of error in these experiments ; a similar mean value is obtained from our measurements which are quoted later.

(b) Deposition below the Decomposition Potential

As was shown some time ago[1], a small quantity of any radioelement deposits even below the decomposition potential and can be measured with the aid of sensitive methods which are now available. Thus, for example, 4 parts per 1000 of RaE are deposited at about −0.17 V in 24 hr on an electrode 1 cm^2 in area when the stirring is thorough ; this deposition is not affected by the presence of foreign ions, apart from bismuth, in the solution. If the solution is made 0.01 N in Bi ions the deposition of RaE no longer takes place. At a higher concentration the percentage deposition should naturally be much smaller ; 4 parts per 1000 of a 0.01 N bismuth nitrate solution would indeed amount to a few milligrams and thus would form a visible cover which cannot exist below the decomposition potential. This specific effect of bismuth ions on the deposition of RaE ions cannot be interpreted in any way other than by replaceability of these isotopes.

We found similar results for ThB, irrespective of whether it was deposited as metal or peroxide[2]. For example, at 1 V i.e. below the deposition potential, 5 per cent deposited and the deposition was in no way affected by the presence of thallium or other ions near to lead. In 10^{-5} N lead nitrate solution, the deposit was already less than ½ part per 1000 and in 10^{-3} N the fraction deposited was no longer detectable. Clearly in this instance also, increased deposition occurs because of the high concentration, but the positions of most of the ThB atoms are taken by lead atoms, depending upon the concentration ratio of the two.

(c) Measurement of a RaD Peroxide Cell

Concerning the question of isotopy of the elements we are mostly limited to indirect methods like those described above, since no single instance is known in which both of two isotopes exist pure and in visible amounts. Visible amounts can be made available only from relatively long-lived elements ; when recovered from minerals they are always contaminated with isotopes, e.g. uranium-II with uranium-I, ionium with thorium, mesothorium with radium, and so on. Radium-D, which occupies a position midway between the long- and short-lived, is always mixed with about ten million times the amount of lead when obtained from pitchblende ; the considerable quantity of radium emanation available to us, however, gave us the opportunity to obtain directly visible amounts of RaD, completely free from lead because of its formation from emanation allowed to disintegrate in carefully purified quartz vessels.

[1] G. Hevesy, *Phil. Mag.* **23,** 628 (1912).

[2] The anodic deposition of ThB at strongly positive potentials was explained by the formation of $(ThB)O_2$ (F. Paneth and G. Hevesy, *Sitz. Ber. Akad. Wiss. Wien* **122,** 1027 [1913]).

In the course of a few weeks the sealed flasks, which had meanwhile become coloured a deep brownish-violet, were opened, washed out with nitric acid which had been distilled through a quartz condenser, and the solution was evaporated. Until completion of the electrolysis care was taken to use only quartz and no glass vessels. According to the conditions of electrolysis metallic RaD or RaD peroxide was obtained as a visible coating on small platinum wires; preliminary experiments allowed this result to be expected since we had convinced ourselves that amounts of lead smaller than 0.001 mgm, as peroxide, are still clearly visible and electromotively effective, i.e. they can be used for building a cell[1]. We have manipulated various quantities of emanation, ½ c on the avarage, but even 100—200 mc are sufficient for carrying out an experiment.

The activity of the wires, as checked by measuring the α- and β-radiation, was of the order of magnitude expected for pure lead-free RaD; moreover, our apparatus was free from lead to the extent that we were able to detect an artificial contamination of 10^{-9} gm Pb.

We measured the electromotive force of the following cell:

$$\underset{10^{-5}N}{Pt/RaDO_2/Ra(DNO_3)_2},\ \underset{10^{-3}N}{HNO_3},\ \underset{satd.}{RaDO_2/KNO_3/KCl},\ \underset{1\ N}{Hg_2Cl_2},\ \underset{1\ N}{Hg}\ \underset{satd.}{}$$

The potential of RaD O_2 was found to amount to -0.884 V. The PbO_2 potential measured in the same conditions was found to be -0.888 on the average[2].

In another series of experiments lead nitrate was added gradually and the following electromotive forces were found (at 20° C) (Table 1).

Table 1

Total normality of the lead isotopes	PbO_2		$(Rad)O_2$	
	εHg (V)	Change in potencial difference	εHg (V)	Change in potential difference
10^{-5}	0.906		0.906	
10^{-3}	0.774	0.032	0.868	0.038
10^{-1}	0.837	0.037	0.839	0.030
	Total change	0.069	Total change	0.068

[1] Refer to J. Koenigsberger and W. J. Müller, *Phys. Z.* **6**, 849 (1905): *Ibid.* **12**, 606 (1911).

[2] The RaD nitrate concentration could only be determined to the nearest order of magnitude and therefore importance should be attached only to the agreement of the two potentials and not to their absolute values.

It is evident that the cells are identical within the limits of experimental error. We attach less importance to this than to the fact that the addition of Pb ions to RaD nitrate solution exercises precisely the same effect on the potential difference of the RaD peroxide which, according to NERNST's theory, the Ra D ions (and only they) should have. This proves that c in the Nernst formula $\left[\varepsilon = \frac{RT}{nF} \ ln \ \frac{c}{C}\right]$ is to be understood as the sum of the concentrations of the isotopic ions present.

A special peculiarity of this RaD peroxide electrode deserves to be mentioned. If it is allowed to remain in contact with air for some time it immediately shows, on immersion, a potential which may be one- or two-tenths of a volt higher than the constant electrode potential established after a certain time. This is probably connected with the strong ionization in the vicinity of the wire.

(d) Experimental Details

The curves described above for the decomposition potential of RaE were obtained as follows : Two gold electrodes, each 1 cm² in area, were immersed in 25 cm³ of 0.1N nitric acid solution and were polarized for a long time until the desired electrode potential had established a constant value. A steady motion of the solution was ensured by passing a current of nitrogen. After the attainment of constant potential a few-tenths of a cubic centimetre of a solution at the same nitric acid concentration and containing radium-E or RaE and Bi was added[1] and the experiment was allowed to run for 24 hr. After this time the electrodes were withdrawn without interrupting the current, washed with distilled water, always in the same way, and measured in an electroscope ; 5 cm³ of the solution were evaporated on a watch glass and likewise measured and hence the percentage deposition of RaE could be calculated. The experiments with ThB also were carried out similarly ; in this case the active solution was added to 100 cm³ of 0.001 N nitric acid and the deposition was made on correspondingly pre-treated platinum electrodes with an area of 4×2 cm². The potential difference was measured by means of a Siemens compensating apparatus.

The RaE solution was obtained directly from emanation, and the thorium-B by exposure of a platinum foil to radiothorium. Particular care was used in the latter case to exclude lead completely ; a part of the experiment was carried out in quartz vessels and with the use of water purified specifically for this purpose[2].

[1] The addition of small amounts of bismuth to make the solution about 10^{-7} N often caused an initial change of the cathode potential by several millivolts.

[2] The purification of the water was that usually employed for determinations of atomic weight (cf. O. HÖNIGSCHMID, *Mitt. d. Inst. Radiumforschung.* **8**, 8).

The effect of adding very small amounts of lead on the deposition of ThB below its decomposition potential was studied as well.

Table 2 clearly shows the decrease in percentage deposition of ThB from a 0.001N nitric acid solution on platinum electrodes (+ 0.4 V, ε_{Hg}) with increasing concentration of lead; in every experiment four electrode surfaces were measured and the mean value was taken.

TABLE 2

Total concentration of Pb isotopes	Amount of ThB deposited, as a percentage of that originally present (%)
5×10^{-12} N.	0.98
10^{-9}	0.75
10^{-7}	0.86
10^{-5}	0.105
10^{-3}	no longer detectable

Thus up to a concentration of the solution of 10^{-7} N, the deposition is only slightly affected, at 10^{-5}N a marked fall is already noticeable, and at 10^{-3}N the deposition is no longer measurable. This method, which can be still further refined by choosing smaller electrodes, still permits the detection of very small amounts of inactive lead, since the addition of another element, e.g. thallium, which is a neighbour of lead, has no noticeable effect on the deposition of ThB even at a concentration of 10^{-3}N Tl.

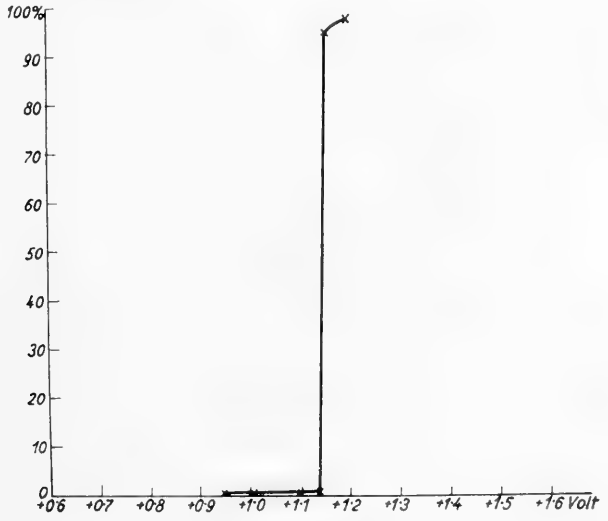

FIG. 4. Anodic precipitation of $ThBO_2$. The lead isotope concentration [Pb + ThB] of the solution 10^{-5}N.

The determination of the decomposition potential by means of the methods mentioned above is based on the assumption that the current strength is large enough to permit deposition of the whole quantity of the radioelement within the duration of the experiment. It is easily seen, e. g. in the electrolysis of a 0.001 N lead nitrate solution, that the above condition is far from being satisfied, since the electrode potential is attained in our apparatus at a current strength of about 3×10^{-6} A which, in the course of 24 hr, is capable of depositing only a very minute fraction of the lead ions present. This is particularly emphasized since, if this point is not taken into consideration, there will be found too high a value in determining the decomposition potential by the methods mentioned (sudden increase in the amount deposited). For example, Fig. 4 shows the apparent decomposition potential of ThB using 0.001 N solution ; it is considerably higher than the calculated value, and the explanation is probably to be found in the reason mentioned above.

We hope to be able to revert to several of the points which have been discussed, particularly to the deposition below the decomposition potential.

4. DISCUSSION

It has already been mentioned above that the difference in the atomic weights of individual isotopic elements exists without any doubt. Hence it follows that, in so far as gravitational properties are concerned, the isotopes are not identical and that by centrifuging, for example, mesothorium should be easier to separate than its isotope radium from barium. On the other hand, a similar differential in the chemical properties of isotopic elements is not observed, we have found replaceability in the electrochemical behaviour. It is concluded that the electrode potential may be written in the form :

$$= \frac{RT}{nF} \ln \frac{\Sigma c}{c}$$

where Σc denotes the total concentration of all the isotopes present, and correspondingly the mass action law may be written in the form :

$$\frac{[\Sigma\text{isotope A}]^{n_1}\ [\Sigma\text{isotope B}]^{n_2} \ldots}{[\Sigma\text{isotope A}']^{n_1'}\ [\Sigma\text{isotope B}']^{n_2'} \ldots} = K$$

The proposition that two atoms with different weights can replace each other in their mass action seems at first glance to contradict the second law of thermodynamics. The contradiction disappears, however, when the concept of chemical individuality, to which the mutual replaceability is related, is considered more closely and is defined appropriately.

We generally ascribe to each element a particular chemical character which varies discontinuously from one element to another. In their mass action silver atoms can replace only other silver atoms and not lead, thallium or other atoms. Accordingly, in the Nernst formula for the electrode potential of silver:

$$\varepsilon = \frac{RT}{nF} \ln \frac{c}{C}$$

ε can be changed only by the addition of silver ions and not by others.

If it is now found that atoms which, in spite of having different atomic weight, replace each other chemically and that c must be understood as the total concentration of the isotopes, it then seems necessary to define the concept of chemical individuality such that this does not imply complete equality of the atoms involved but the mutual replaceability of the two atoms. The correlative of replaceability seems to be equality of the nuclear charge numbers whose fundamental importance becomes more and more prominent.

Summary

Experiments have been made to discover whether isotopic elements can replace each other chemically; the following electrochemical methods have been employed for this purpose.

(1) The electrolytic deposition of radium-E with and without the addition of bismuth has been studied and it has been found that the decomposition potential is displaced by the addition of bismuth in the sense and by the amount which would be expected of the addition of the same (RaE) ions in accordance with NERNST's theory; a study of the deposition of thorium-B with and without the addition of lead yielded the same result.

(2) It has been shown that the deposition of the very small amounts of radioelements which precipitate below the decomposition potential is hindered by the presence of isotopes (and only by these), and this likewise can be explained only by replaceability.

(3) Radium emanation has been allowed to disintegrate in quartz and the radium-D formed has been deposited electrolytically as the peroxide on platinum wires; visible and at the same time electromotively active amounts (a few-thousandths of a milligram) have thus been prepared. The cell $RaDO_2 \mid RaD(NO_3)_2 \mid KNO_3 \mid KCl,Hg_2Cl_2,Hg$ showed the same electromotive force as a cell similarly made with lead peroxide, and furthermore the addition of lead ions to the RaD solution changed this e. m. f. in the same way a corresponding addition of RaD ions should change it according to NERNST's theory; hence it is concluded that the ionic concentration c in the Nernst formula

$$= \frac{RT}{nF} \ln \frac{c}{C}$$

must be understood as the sum of the isotopic ions.

From our study, therefore, the conclusion must be drawn that isotopic elements are able to replace each other in their mass action.

Comment on paper 6

As shown by Nernst the electrode potential of a metal is proportional to the logarithm of the concentration of its ions present in the surrounding solution. The validity of this regularity was tested up to 0.0001 N ionic concentration. The application of labelled bismuth and labelled lead permitted us to demonstrate the validity of this regularity at much lower ionic concentrations than the above mentioned one. When this investigation was carried out in 1913 the notion of isotopes had just emerged, and it was thus of interest to demonstrate that the voltage at which RaE, for example, is precipitated on the cathode, is influenced by the addition of a bismuth salt to an extent to be expected on assuming the practical chemical identity of bismuth and RaE. It is not, however, influenced by adding salts of other metals. Below its decomposition voltage minute traces of RaE are deposited as well, this minute precipitation is also influenced by addition of bismuth salts, but not by addition of salts of other metals.

The large amount of radon available at the Vienna Institute made it possible to obtain a visible RaD layer on a platinum wire. The electrode potential of a $RaDO_2$ electrode was found, measured against a calomel electrode undistinguishable from the potential of a lead peroxyde electrode. The experiments were carried out with peroxide of lead instead of metallic lead, as the electrode potential of metallic lead was found not to be sufficiently reproduceable.

If it were possible to measure these electrode potentials to an accuracy of several decimals, we would presumably measure some difference between the electrode potential of lead peroxide and radium D peroxide, as isotopes are not strictly identical in their chemical properties. The very far-reaching practical chemical identity of isotopes of an element is, however, conspicuously demonstrated by the results of this paper.

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7. THE VELOCITY OF DISSOLUTION OF MOLECULAR LAYERS

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The velocity of dissolution of finite layers can be followed quantitatively by considering the process of dissolution as being comprised of two partial processes; one of these consists in the formation of a layer of saturated solution surrounding the solid surface and the other is a process of diffusion from this boundary layer into the liquid[1].

The velocity of dissolution is represented by the equation:

$$dx/dt = DOF(c_0 - c)/\delta$$

where δ denotes the thickness of the boundary layer, F a proportionality factor, D the diffusion coefficient, O the area, c_0 the saturation concentration, and c the concentration of the solution.

The dissolution will therefore proceed more rapidly the smaller the thickness of the boundary layer, i.e. the greater the speed of stirring, the greater the diffusion velocity of the participating molecules and the further the solution is from the saturated state; the formula also shows a parallelism between solubility and velocity of dissolution.

Nernst and Brunner[2] have shown that these ideas are quite generally applicable to heterogeneous reactions.

The present communication discusses the course of the dissolution process of molecular layers, which could be also described as infinitely thin, and the extent to which the above simple equation is satisfied.

THE PREPARATION OF INFINITELY THIN LAYERS

It is well known that an infinitely thin layer of radioactive metal or its oxide, known as the so-called active deposit, can be obtained simply; by the decay of the gaseous emanations metallic products are formed, which are isotopes of polonium, lead, bismuth and thallium and which

[1] Noyes and Whitney, *Z. phys. Chem.* **23,** 689 (1897).
[2] Nernst and Brunner, *Z. phys. Chem.* **47,** 52, 56 (1904).

gradually become deposited from the suspension in the air; this deposition process can be considerably accelerated by applying an electric field. In our experiments we made use of a radiothorium preparation, which provides a constant source, and the active deposit yielded by the emanation was collected on a quartz surface 1.6 cm in diameter. The quartz disk was covered with a mixture of ThB (lead isotope) and ThC (bismuth isotope) because the first decay product of emanation, ThA, decays very quickly with a half-life of $^1/_7$ sec. A simple calculation yields 5×10^{-11} gm as the total mass of the deposit, of which about 90 per cent consists of ThB and 10 per cent of ThC. In order to cover the surface completely with a molecular layer of lead 2×10^{-6} gm would be necessary, i.e. 50,000 times the amount actually present; we can thus rightly consider the surface as having an infinitely thin covering of lead and bismuth.

The velocity of dissolution was determined as follows: The quartz disk was allowed to stand for several hours after cessation of the activation until radioactive equilibrium had been established and the β-activity formed by the deposit, and showing the relative amounts of Pb and Bi present, was then determined; the disk was then placed in a bell-shaped vessel, provided with an outlet tube and cock, and after a certain time the 100 cm^3 of liquid in the vessel was drained out. Care was taken to attain a constant stirring speed, the disk being placed in the solution only when this speed had been established.

The β-activity of the quartz disk treated in this way was then measured again 15 min after completion of the experiment and at various later time intervals, and it was thus possible to decide upon the amounts of Bi and Pb present, from the change of the activity with time, and to determine the percentage which had entered into solution.

THE DETERMINATION OF THE VELOCITY OF DISSOLUTION

The investigation was concerned with the determination of the effect

(1) of the concentration of acid in the solution
(2) the viscosity
(3) of the speed of stirring
(4) of the time

on the velocity of dissolution of the molecular layer and finally on the effect due to isotopes of the corresponding elements in the solution.

The percentages of Bi and Pb isotopes which dissolve in nitric acid in 60 sec, under the same experimental conditions, are summarized in Table 1.

TABLE 1. — AMOUNTS DISSOLVED IN 60 sec

	In water (%)	10^{-5} N HNO_3 (%)	10^{-4} N HNO_3 (%)	10^{-3} N HNO_3 (%)	10^{-2} N HNO_3 (%)	10^{-1} N HNO_3 (%)	N HNO_3 (%)
Bismuth isotope (ThC)	37	38	35	61	72	77	78
Lead isotope (ThB)	60	61	60	80	81	83	84

The velocity of dissolution is the same in 10^{-4} N acid as in conductivity water but increases with further increase of the acid concentration and in N acid amounts to about twice the above value. It is well known that the bismuth isotopes dissolve colloidally in water and from diffusion experiments the conclusion was drawn that this is no longer the case in 10^{-3} N acid[1]. It suggests itself to associate the sudden increase in the velocity of dissolution with this change.

The velocity of dissolution of finite layers depends on, among other factors, the diffusion velocity of the products involved. In order to change this velocity glycerol was added to the nitric acid in order to cause a considerable increase of the viscosity and a corresponding lowering of the diffusibility of the hydrogen and other ions without otherwise changing the experimental conditions. The consequence of the glycerol addition was, as is clear from Table 2, a decrease in the velocity of dissolution.

TABLE 2

	Dissolved in 10^{-3} N HNO_3	Dissolved in 10^{-3} N HNO_3 containing 25% glycerol
Bismuth isotope (%)	61	52
Lead isotope (%)	80	73

The viscosity of the glycerol mixture found by the ordinary outflow method was 1.650 relative to that of water as unity.

Even after treatment for several hours with concentrated acids it is found that the quartz disk still retains about 20 per cent of its original ThB—ThC coating which, however, is not present on the surface but is situated inside the quartz where it has arrived through the so-called

[1] F. PANETH, *Kolloid-Z.* **13,** 1, 297 (1913); G. HEVESY, *Phys. Z.* **14,** 1209 (1913).

radioactive recoil process. A portion of the active deposit is laid down on the quartz disk in the form of ThA, the ThA then emits α-particles with the result, according to the principle of action and reaction, that a recoil of the atoms is required and thus some of the ThB atoms formed from ThA are deposited under the surface of the quartz. The range of such recoil atoms amounts to about $^1/_{10}$ cm in air and, therefore, less than 10^{-4} cm in quartz; this thin layer of quartz is quite sufficient to protect the fraction driven inward by the recoil effect from the reaction of the acid, although radiation, from which its presence can be inferred, still affects the electroscope through this layer.

The portion of the active deposit found underneath the surface depends on the time and other conditions of exposure which have been chosen to be strictly the same in all these experiments. The portion of the active deposit occurring below the quartz surface, found experimentally to be 20 per cent, was not taken into account in compiling the tables, the values in which refer only to the soluble part of the active deposit.

The determination of the effect of the stirring speed on the velocity of dissolution meets with difficulties. Because of the large velocity of dissolution of molecular layers the times of experiment must be limited to a few minutes and the unavoidable immersion and withdrawal of the quartz disk from the solution always acts as intense stirring. In our experience the effect of stirring velocity on the velocity of solubility of molecular layers was not considerable.

The Relation between Velocity of Dissolution and Solubility

As shown by the above formula, the velocity of solubility of finite layers increases with the solubility of the substance; this is true also for infinitely thin layers, and thus the velocity of solubility of the lead isotopes is greater than that of the bismuth isotopes (Table 1), both in water and in nitric acid, corresponding to the greater solubility of the lead salts involved.

Lead peroxide dissolves more slowly than metallic lead and lead monoxide, in agreement with its lower solubility in HNO_3. Such peroxide layers[1] were produced by the anodic deposition of $ThBO_2$ on platinum. Only 20 per cent of $ThBO_2$ dissolved in the same conditions in which 80 per cent ThB entered the solution. Since dissolution from quartz is not strictly comparable with dissolution from platinum, a comparison was therefore made between the velocity of solubility values of $ThBO_2$ and ThB likewise deposited on platinum by a cathodic reaction; in this case also more of the latter dissolved as is proved by the figures in Table 3.

[1] F. Paneth and G. Hevesy, *Wien. Ber.* **122,** 1038 (1913).
[2] F. Paneth and G. Hevesy, *Wien. Ber.* **123,** 1050 (1913).

Table 3

Ratios of the amounts of ThB and $ThBO_2$ dissolving in 1 min		Ratios of the amounts of ThC and $ThCO_2$ (?) dissolving in 1 min	
$10^{-2}N$ HNO_3	$10^{-2}N$ HNO_3 + $10^{-1}N$ oxalic acid	$10^{-2}N$ HNO_3	$10^{-2}N$ HNO_3 + $10^{-1}N$ oxalic acid
4.0	0.76	3.1	0.75

It is also evident that in the presence of a reducing agent such as oxalic acid the large difference between the dissolution velocities of the cathodically and anodically deposited lead isotopes disappears, in agreement with the ready solubility of the peroxide in oxalic acid solution.

In the more recent development of electrochemistry particular attention is devoted to the reactions which take place between the deposited products and the electrode material; the study of the velocity of solubility with the electrolytically deposited radioelements offers an easy method of approaching more closely to these problems; thus, in the case of polonium it was proved that this element forms stable compounds more easily with Pt and Pd than with gold.

Change in the Dissolution Velocity due to the Presence of Isotopic Ions of the Dissolving Metal in the Solution

Isotopic atoms are interchangeable in their electrochemical reactions[1]. Now the dissolution of a metal is to some extent the reverse of its electrolytic deposition and therefore it is to be expected that ThB will dissolve only to the same small extent as lead in nitric acid saturated with lead nitrate.

Before discussing the behaviour of a molecular layer when dissolving in a solution which already contains some dissolved isotope, we would like first to explain in more detail the process which takes place between a solid phase and its saturated solution. Just as the equilibrium state between a liquid and its saturated vapour is regarded as dynamic, i.e. the assumption is made that the same number of molecules condense from the vapour and leave the liquid in unit time, the equilibrium state between a solid phase and its saturated solution is also regarded as dynamic, i.e. it is assumed that at the boundary of, for example,

$$PbCl_2 \text{ solid} \mid \text{water saturated with } PbCl_2$$

a dynamic exchange of $PbCl_2$ molecules takes place between the two

[1] G. Hevesy and F. Paneth, *Phys. Z.* **15**, 797 (1914).

phases. It is necessary to know the rate of this exchange because if it is very large the $(ThB)Cl_2$ molecules in the solid phase will exchange directly with the $PbCl_2$ molecules of the solution and thus simulate direct dissolution.

An answer to this question would be possible if, for example, a saturated lead chloride solution could be shaken with solid $PbCl_2$, the lead atoms of which were numbered or characterized in any other way without affecting their chemical properties, by finding in which phase the numbered atoms then existed. Such ideally labelled lead atoms are the radioactive isotopes (ThB, RaB, AcB, RaD, etc.). We need only add, for example, $(ThB)(NO_3)_2$ to the solution of lead nitrate, precipitate the Pb—ThB mixture as chloride and thus to obtain a "coloured" lead chloride. If 1 mgm of $PbCl_2$ were originally associated with one relative unit the detection of this relative unit in the saturated solution would allow the inference that 1 mgm of the lead atoms originally in the solid phase had then been transferred into the saturated solution, or vice versa.

The application of radioactive indicators thus serves to permit tracing of the exchange of atoms of the same kind between two phases; the "colouring" of the labelling atoms is a purely radioactive property and, although their mass is different from that of the labelled isotope (they have different atomic weights), their chemical reactions, with which we are concerned, are still the same[1].

To determine the velocity of exchange between the solid lead chloride and its saturated solution, 250 mgm of ThB-labelled $PbCl_2$ was shaken with 25 cm^3 of a saturated solution of pure lead chloride in a thermostat at 20°C. After 24 hr the mean value of ten experiments showed that 1.2 mgm, or $\frac{1}{2}$ per cent of the lead chloride originally in the solid phase had entered the saturated solution; after 48 hr the value was about $\frac{3}{4}$ per cent. The lead chloride solution was prepared by cooling an originally slightly supersaturated solution in the thermostat and was therefore fully saturated; the possible sources of error all tended to yield high values for exchange and the above value should therefore be regarded as an upper limit only.

The velocity of exchange depends very markedly on the mechanical consistency of the solid phase, and this also applies to the velocity of solubility. It is thus imperative to compare these two quantities under the same experimental conditions. For example, on shaking 250 mgm

[1] These ideas do not apply strictly to diffusion processes or, therefore, to the exchange of atoms in the same phase, because the velocity of diffusion is dependent on the mass; this dependence is very slight, however. Considered from the standpoint of diffusion only those atoms which are both isotopic with and of the same mass as those under study are really ideal indicators. It appears, however, that UY may be such an ideal indicator for UX_1.

of the same lead chloride 44 per cent had already dissolved in 1 hr, representing 44 per cent of the saturation concentration since the liquid volume amounted to 25 cm^3 and 250 mgm are soluble in this volume.

It is seen, therefore, that the velocity of exchange between the two phases is small compared with the velocity of solubility. It will be seen later that in the case of a molecular layer the exchange velocity becomes much larger when expressed on a percentage basis but is still smaller than the velocity of solubility, and thus a diminution of the velocity of dissolution of a substance can still be detected by the presence of its isotope in the solution.

To this end we have compared the amounts of ThB collected on quartz which dissolved in water and in a saturated $PbCl_2$ solution, under the same conditions, in 1 hr. There was only a small difference since in the first case 79 per cent dissolved while in the second the value was 75 per cent.

Shorter experimental times were then chosen and thus the presence of lead ions in the solution had a very considerable effect on the velocity of solubility of ThB. These experiments were performed with the same apparatus used for obtaining the values recorded in Table 1.

TABLE 4. — EXPERIMENTAL TIME 60 sec

	Amount dissolved in 10^{-3} N HNO_3 (%)	Amount dissolved in 10^{-3} N HNO_3 saturated with $Pb(NO_3)_2$ (%)
Bismuth isotope ThC ..	61	64
Lead isotope ThB	80	67

The presence of lead ions in the solution diminishes the velocity of solubility of the lead isotope but not that of the bismuth isotope. It is a fortunate circumstance that ThB and ThC are simultaneously present in the same place on the quartz surface, and when, in spite of this, the velocity of solubility of only one is affected, this means that there is a specific effect due to the addition of the appropriate element; for example, glycerol, which has no selective effect but which increases the viscosity of the solution, affects the velocity of solubility (Table 1) of ThB and ThC equally.

In order to ascertain whether a small concentration of lead ions affects the amounts of ThB and ThC dissolved, we have performed experiments in 10^{-3}N HNO_3 solutions which were also 10^{-3}N in lead. The time of experiment was 40 sec and the arrangement was different from that described above. The ratio of the amounts of Pb and Bi dissolved was found to be :

in pure 10^{-3}N HNO_3	1.99
in 10^{-3}N $Pb(NO_3)_2 + 10^{-3}$N HNO_3	1.49

which is still a significant difference.

The conclusion to be drawn from these experiments is that the velocity of exchange between the solid phase and its saturated solution is already commensurate with the velocity of dissolution for a molecular layer, but, that when the experimental time is short an effect on the velocity of dissolution due to the presence of isotopic ions in the solution can be detected.

The following experiment seemed to be of interest in connection with those described above: 200 mgm of $Pb(NO_3)_2$ labelled with ThB was added to a solution of $PbCl_2$ (200 mgm), a portion of the $PbCl_2$ was then allowed to crystallize out and the distribution of the different kinds of lead atoms between the chloride and nitrate was studied. After a few minutes required for performing the manipulations it was shown that there was a completely uniform distribution of all the lead atoms, within the limit of error amounting to 1 per cent.

Z. Klemensiewicz[1] has recently performed similar experiments. He studied the distribution of ThB and also RaB between a lead amalgam and a mercuric nitrate solution and found a completely uniform distribution; the accuracy of his experiments was greater, with an error of ½ per cent.

Summary

The velocity of dissolution of molecular (infinitely thin) layers shows qualitatively the same behaviour as that of finite layers. The velocity of dissolution of lead and bismuth isotopes in nitric acid increases with acid concentration, with lowering of the viscosity of the solution and with the solubility of the substance involved.

The presence of lead ions in the solution lowers the velocity of dissolution of the lead isotope ThB without affecting that of the bismuth isotope ThC.

The velocity of exchange between the molecules of solid lead chloride and a saturated lead chloride solution can be determined by labelling the lead chloride with ThB; in the case of a finite layer it is vanishingly small compared with the velocity of dissolution but in the case of a molecular layer the two properties are commensurable.

[1] Z. Klemensiewicz, *C. R. Acad. Sci., Paris* **158**, 1889 (1914).

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8. THE EXCHANGE OF ATOMS BETWEEN SOLID AND LIQUID PHASES

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When a liquid is in contact with its saturated vapour there will take place, in accordance with kinetic ideas, a constant exchange between the molecules in the two phases. Correspondingly, it is to be expected that a kinetic exchange of the molecules will likewise occur when a solid phase is in contact with its saturated solution.

Radiochemical methods permit an experimental study of this exchange. For example, if the exchange between the molecules of a solid layer of lead chloride and a saturated solution of lead chloride is to be determined the following procedure is adopted: A known amount, in relative (electroscopic) units, of ThB is added to a solution of known $Pb(NO_3)_2$ content and the whole is precipitated with hydrochloric acid. In accordance with all previous experience the ThB can no longer be removed chemically from such a mixture of $PbCl_2$ and $ThBCl_2$; if there is, for example, one atom of ThB mixed with 10^{10} lead atoms on the average in this mixture, this ratio will remain the same after any chemical operation and if a ThB atom can be detected electroscopically in the lead chloride phase which was previously free from ThB the conclusion can be drawn that 10^{10} of the lead atoms originally mixed with the ThB have also entered this phase. Thus the ThB or another isotope of lead serves as an "indicator" for lead.

If solid lead chloride labelled with ThB is shaken with a saturated (unlabelled) solution of lead chloride for 36 hr at 20°C it is found that less than ½ per cent has been transferred from one phase into the other.

The determination of the velocity of dissolution of lead chloride having the same grain size showed that 44 per cent of the amount of $PbCl_2$ corresponding to saturation passed into solution within 1 hr; since the number of exchanged molecules (expressed as a percentage of all those present) is extremely small it appears that the velocity of exchange of lead chloride molecules between solid lead chloride and the saturated solution of $PbCl_2$ is vanishingly small compared with the velocity of dissolution of solid lead chloride.

A different result is obtained, however, if a study is made of the exchange, not between a finite layer of lead chloride and its saturated solution but between a molecular film of lead chloride, which can easily be prepared by a radiochemical method, and the saturated solution. It is then seen that the percentage of exchanged molecules is very considerable and the velocity of exchange becomes commensurable with the velocity of dissolution of the molecular layer.

This result can be expected from kinetic considerations; a rapid exchange can take place, in general, only in the superficial layers.

A lead rod, 4 cm long and 5 mm in diameter, was immersed for 1 min in 10 cm^3 of a lead nitrate solution labelled with a known amount of ThB, and then the quantity of ThB deposited on the lead surface was determined. This gives the number of lead ions originally in the solution which have thus been transferred to the lead surface. The first column of Table 1 records the normality of the $Pb(NO_3)_2$, the second the number of lead ions per thousand originally in the solution and then occurring on the surface of the lead, the third the amounts of lead in grammes, the fourth the amount, expressed as a fraction, of that required, according to MÜLLER and KOENIGSBERGER[1], to indicate the potential of lead peroxide. On the basis of the Loschmidt number the mass of a unimolecular layer of PbO_2 is calculated[2] as 3.2×10^{-7} gm. According to the measurements of KOENIGSBERGER and MÜLLER[2] twice this mass is required for optical detection and eight times to impart the PbO_2 potential to an area 1 cm^2 in extent.

TABLE 1

Normality of the solution in $Pb(NO_3)_2$	Promille of lead of the solution transferred to the solid phase	Amount of lead exchanged (gm)	Number of molecular layers 1 cm^2 in area which can be covered by the amount of exchanged lead
10^{-5}	4.3	4.4×10^{-8}	0.069
10^{-4}	4.5	4.6×10^{-7}	0.72
10^{-3}	3.9	4×10^{-6}	6.2
10^{-2}	3.7	3.8×10^{-5}	59
10^{-1}	1.7	1.7×10^{-4}	266
1	0.4	0.4×10^{-4}	625

According to our ideas on the process of the galvanic production of current, lead will either go into solution or will be deposited, when a lead rod is immersed in a solution of lead nitrate, according as the concentration of lead nitrate is on one or the other side of the limit at which

[1] cf. W. J. MÜLLER and J. KOENIGSBERGER, *Phys. Z.* **6,** 849 (1905).
[2] J. KOENIGSBERGER and W. J. MÜLLER, *Phys. Z.* **12,** 606 (1911).

a potential difference of zero prevails between the lead nitrate solution and the metallic lead (absolute zero point of the electrolytic potential). Thus it might be thought that the amount of lead deposited in the above experiments does not represent the exchange between the two phases but is the result of such an unbalanced electrolytic process. The calculation of the amount which can be expected to be deposited at an isolated electrode shows, however, that the value is much smaller than that actually observed.

The capacity of the double layer at the metal-electrolyte boundary, from the measurements of KRÜGER and KRUMREICH[1], amounts to 27 μF ; the potential difference of this condenser in the case of $Pb/Pb(NO_3)_2$ is always less than $\pm$ 0.2 V ; thus the calculated charge of the condenser is 5.4×10^{-6} C. This quantity of electricity corresponds to 5.6×10^{-9} gm Pb, which is considerably less than the deposit of lead found by experiment.

In order also to confirm experimentally that an exchange of atoms between the two phases, and not a one-sided deposition, is involved, the following experiments were performed :

A lead rod similar to those used in the experiments already mentioned was coated electrolytically with a layer of metallic lead labelled with ThB, immersed in 10 cm^3 of lead nitrate solution for 1 min and then, by determining the ThB content of the solution, the number of lead atoms transferred from the metallic phase into the lead nitrate solution was determined.

Thus it was possible to establish that while 1.7×10^{-4} gm had deposited from 10 cm^3 of a 0.1 M lead nitrate solution on an area of 2 cm^2 in 1 min., 1.6×10^{-4} g Pb had correspondingly entered the solution in identical conditions in the experiment just mentioned.

In our experiments, therefore, there is indeed an exchange of atoms between the metallic phase and the lead nitrate solution. Because of the magnitude of the amounts exchanged, which in certain conditions amount to one hundred times the unimolecular layer, the process cannot be a pure "kinetic" exchange (exchange at complete thermodynamic equilibrium) but involves nonuniformity of the lead surface and precipitation of the lead atoms at particular points from the solution. The observed exchange is essentially a result of "local currents".

The velocity with which the exchange of the lead atoms takes place in the interior of the solid metallic phase can be computed approximately since it is equal to the velocity of diffusion of lead in solid lead.

The diffusion velocity of lead in mercury according to M. v. WOGAU[2] amounts to 0.6 cm^2 hr^{-1} at 18°C ; in solid lead the value is many times

[1] KRÜGER and KRUMREICH, *Z. Elektrochem.* **19,** 620 (1913).
[2] M. v. WOGAU, *Ann. Phys.* **23,** 345 (1907).

less since the viscosity of solid lead is very much greater than that of liquid mercury[3]. The viscosity of mercury is 0.016 at 18°C whereas the value for solid lead, according to KURNAKOW and ZEMACZNY[4], is 3×10^{12}; the velocity of diffusion of lead in solid lead calculated from these figures is 2×10^{-14} cm^2 hr^{-1}. The minuteness of this diffusion velocity is best brought out by means of the following analysis: Consider a diffusion cylinder consisting of four equal parts, each part being surrounded by four molecular layers with an assumed thickness of 0.8×10^{-7} cm and with the lead atoms of the lowest four molecular layers labelled. After 1 hr there will be less than one per thousand of the labelled lead atoms in the uppermost part of the diffusion cylinder, in the third part only 1.6 per cent. The kinetic exchange during 1 min can extend only to the uppermost molecular layer and to a small extent to the second and third layers.

Table 2 records the amounts of lead which have exchanged between a 0.001 N $Pb(NO_3)_2$ solution and a lead rod in various times.

TABLE 2

Time (sec)	Amount of lead exchanged (gm)	Number of molecular layers 1 cm^2 in area which the exchanged lead can cover
15	2.0×10^{-6}	3.1
15	2.3×10^{-6}	3.3
30	2.8×10^{-6}	4.4
30	2.8×10^{-6}	4.4
60	3.5×10^{-6}	5.5
60	3.6×10^{-6}	5.6

Table 3 contains the results of such experiments in which a lead rod dipped into 10 cm^3 of a 0.1 N solution of $Pb(NO_3)_2$.

TABLE 3

Time (min)	Amount of lead exchanged (gm)	Number of molecular layers 1 cm^2 in area which the exchanged lead can cover
1	1×10^{-4}	156
10	2.1×10^{-4}	328
30	3.2×10^{-4}	564

[3] How far such an extrapolation is permissible for the solid state will shortly be discussed on the basis of experiments.

[4] KURNAKOW and ZEMACZNY, *Jb. Radioakt.* **11**, 25 (1914).

Different behaviour is found in studying lead peroxide surfaces immersed in a lead solution labelled with ThB. In this case the exchange is much less; between a PbO_2 surface 2 cm² in area and 10 cm³ of a 0.001 N $Pb(NO_3)_2$ solution, containing 0.001 N HNO_3 and saturated with similarly labelled PbO_2, the following exchange takes place:

TABLE 4

Time	Amount of lead exchanged (gm)	Expressed as molecular layers 1 cm² in area	Expressed in fractions of the amount required to impart the PbO_2 potential to the area of 1 cm²
10 sec	1.6×10^{-7}	$^1/_4$	$^1/_{32}$
1 min	2.4×10^{-7}	$^1/_3$	$^1/_{24}$
10 min	1.0×10^{-6}	1.5	$^1/_5$
60 min	2.0×10^{-6}	3.1	$^3/_8$

TABLE 5. — THE TIME OF EXPERIMENT IN THIS CASE IS ALWAYS 1 min; THE CONCENTRATION OF THE LABELLED LEAD NITRATE SOLUTION VARIES BETWEEN 10^{-1} AND 10^{-6} N

Normality of the solution of lead nitrate	Amount of lead exchanged (gm)	Expressed in molecular layers 1 cm² in area	Expressed in fractions of the amount required to impart the PbO_2 potential to the area of 1 cm
10^{-6}	0.64×10^{-8}	$^1/_{10}$	$^1/_{80}$
10^{-5}	4.0×10^{-8}	$^1/_6$	$^1/_{48}$
10^{-3}	3.0×10^{-8}	$^1/_2$	$^1/_{16}$
10^{-1}	2.2×10^{-7}	3.5	$^1/_2$

Here also an exchange rather than a unilateral dissolution is involved as is proved by the following experiments: This time a labelled PbO_2 surface 2 cm² in area is immersed in 10 cm³ of a 0.001 N $Pb(NO_3)_2$ solution, saturated with PbO_2 and containing 0.001 N HNO_3, and it is found that the following amounts of lead (Table 6) have passed into solution from the solid phase:

TABLE 6

Time	Amount of lead exchanged (gm)	Expressed in molecular layers 1 cm² in area	Expressed in fractions of the amount required to impart the PbO_2 potential to the area of 1 cm²
10 sec	1.5×10^{-7}	$^1/_4$	$^1/_{32}$
1 min	2.8×10^{-7}	$^1/_3$	$^1/_{24}$
20 min	0.8×10^{-6}	1.2	$^1/_6$

The experiments just described are made difficult owing to the breaking off of invisible amounts of lead peroxide which fall into the solution

and are co-determined when the solution is evaporated, thus producing an erroneously high exchange.

In the experiments discussed here it was merely assumed that lead and ThB cannot be separated by chemical and electrochemical reactions, as was first proved by FLECK and later confirmed by many authors. If one phase contains on the average 10^{10} atoms per atom of ThB and if we can detect a ThB atom in the other phase which was originally free from ThB then, as already mentioned in the introduction, the conclusion can be drawn that 10^{10} lead atoms also have been transferred from the first to the second phase. Our experiments do not indicate how many atoms have changed places more than once between the two phases.

It should be mentioned that when diffusion processes are involved the presence of one ThB atom cannot strictly be taken to imply the accompaniment by 10^{10} atoms of lead, since the diffusion velocities of ThB and Pb are not equal. As far as solid and liquid phases are concerned, however, in which the diffusion velocity is very little dependent on the mass, it is practicable to draw the above-mentioned conclusion and, for example, to equate the velocity of diffusion of lead isotopes in lead to that of lead in lead.

Summary

The exchange of atoms between two phases, for example, between metallic lead and a lead nitrate solution, can be followed by labelling the lead in one phase with one of its isotopes, for example, with ThB; the amount of labelled lead transferred in a given time into the other phase can then be determined.

In the case of $Pb/Pb(NO_3)_2$ the exchange is very rapid and depends mainly on the local currents. At particular points in the metal some lead goes into solution and at other places lead is deposited from the solution.

The exchange between a surface of lead peroxide and a lead nitrate solution is much less ; in the experimental conditions described in the paper it amounts to only one-third of a molecular layer of lead peroxide in a 0.001 N solution during the course of 1 min. The whole molecular surface layer is replaced only after 1 hr has passed.

In using stable lead peroxide the ideal case of kinetic exchange is much more nearly approached — exchange with complete thermodynamic equilibrium between the two phases — than when metallic lead is used.

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9. THE INTERMOLECULAR EXCHANGE OF ATOMS OF THE SAME KIND

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The present study is intended as a contribution to the answer of the question as to whether and when interchange of similar atoms takes place within a molecule and also between neighbouring molecules of like or unlike kinds. In considering a benzene molecule, for example, the question arises as to whether a carbon or hydrogen atom can move by exchanging places with another similar atom from one position to another in the benzene hexagon, or whether a certain hydrogen atom is always bound to the same carbon atom. If two neighbouring benzene molecules are considered there is the further question as to whether carbon or hydrogen atoms which were originally present in the first molecule may or may not be found in the second molecule after a definite time.

Such an exchange of positions could be produced either directly by the atoms vibrating within a molecule periodically entering into the sphere of attraction of another molecule, or indirectly in the following way : If there is dissociation such that a hydrogen atom splits off from each of two benzene molecules the dynamic nature of the dissociation process in which the atom is recaptured yields a 50 per cent chance that the hydrogen atom which originally was separated from the first molecule will enter the second molecule and thus be subjected to an exchange of position. Because dissociation and recombination processes take place very rapidly[1], even the slightest dissociation in the liquid phase, where molecular collisions occur extremely often, will lead to such exchange in a short time.

Although this question cannot be decided by experiment with benzene, yet this can be done with lead compounds, for example, by means of radioactive methods. It is well known that there are different isotopes of lead which can be distinguished easily and with certainty through their radioactive properties, although they exhibit the same chemical behaviour. By preparing two different compounds of lead, the one from

[1] M. Le Blanc and K. Schick, *Phys. Chem.* **46,** 213 (1903).

ordinary and the other from radioactive lead, it is possible to distinguish any atom of lead in the one substance from any atom of lead in the other since they have distinct properties. By dissolving the two compounds and after a certain time separating them again a simple measurement of radioactivity will show whether each atom of lead is still in the same kind of molecule as before the experiment or whether an exchange of atoms has taken place.

It has previously been demonstrated[1] that when equimolecular quantities of inactive lead chloride and active lead nitrate are dissolved and the latter subsequently recrystallized half of the active lead atoms originally present in the nitrate molecules transfer to the lead chloride. The originally inactive lead chloride was proved to be half as radioactive after the experiment as the lead nitrate was before.

The same result was obtained with the following combinations : Lead nitrate (active) and lead chloride in pyridine ; lead formate (active) and lead acetate in water ; lead acetate (active) and lead tetra-acetate in glacial acetic acid ; and lead tetra-acetate (active) and lead acetate in glacial acetic acid.

In contrast it was found that there is no exchange of lead atoms when the lead is firmly bound to carbon. The behaviour of organically bound lead is illustrated by the following examples : Lead chloride (active) and tetraphenyl lead dissolved in pyridine ; lead acetate (active) and tetraphenyl lead in amyl alcohol ; and lead nitrate (active) with diphenyl lead nitrate in dilute ethyl alcohol.

The original activity or inactivity of the dissolved substances in these instances was not modified by the experiment. The conclusion to be drawn from these findings is that exchange of atoms does not take place even if the lead is undissociated in only one of the two compounds. Exchange is even less likely when this type of binding of the lead exists in both components and particularly when these components are chemically identical, in other words, when they are molecules of the same substance. There will be no exchange of lead atoms between two molecules of tetraphenyl lead. The results up-to-date suggest, therefore, that an intermolecular exchange of atoms (at least in the time required to perform chemical operations) is connected with the existence of an electrolytic dissociation.

The existing experimental data are inadequate to prove whether two similar atoms of the same molecule are able to exchange in a measurable time, although it can be assumed probable that the opportunities for positional exchange within one molecule are similar to those arising between two neighbouring molecules. We intend to attack this problem more closely by introducing a radioactive and an inactive lead atom into

[1] G. Hevesy and E. Rona, *Phys. Chem.* **89,** 303 (1915).

the same molecule but with different bonding. A further range of application for our method might be opened up by splitting off one of the two lead atoms and making comparative measurements of radioactivity on the products.

EXPERIMENTAL

The radioactive lead was prepared in the following way : The active deposit of a strong radiothorium preparation, which had been recovered from a mesothorium sample whose activity (gamma) corresponded to 5 mgm of radium, was collected on the surface of a negatively charged lead foil. The activated lead was then dissolved in nitric acid and the resulting nitrate was converted as required into compounds such as chloride, formate, acetate, etc. The salts obtained in this way were labelled radioactively with the lead isotope thorium-B.

The activity was measured in the usual way with the aid of an α-electroscope. The substance to be measured was spread on a metallic surface and its activity compared with that of a control substance of the same weight and identical surface conditions.

1. Lead Nitrate (Active) and Lead Chloride in Pyridine

An amount of chloride (0.76 gm) and 0.90 gm of nitrate were dissolved completely in 100 gm of boiling pyridine and the solution was kept hot for $\frac{1}{4}$ hr. The lead chloride which crystallized on cooling was filtered at the pump, washed with a little cold pyridine and with ether to remove the solvent, and dried in a vacuum. The sample was quite free from nitrate. Chloride prepared from the original nitrate was used as a standard.

Measurement : 0.268 gm of substance caused the following ionizations : Experimental sample, 2.76 scale divisions per min (calculated for complete exchange $\frac{1}{2} \times 5.64 = 2.82$ scale divisions per min). Standard sample, 5.64 scale divisions per min.

2. Lead Formate (Active) and Lead Acetate in Water

A quantity of lead formate (3.00 gm) and 3.83 gm of sugar of lead (Kahlbaum) were dissolved in 25 ml hot water. After keeping warm for $\frac{1}{4}$ hr the majority of the sparingly soluble lead formate was crystallized by cooling the solution ; the crystals were filtered at the pump, freed from traces of adhering acetate by washing with alcohol and dried in a vacuum. A sample of the active lead formate was used as a standard. Measurement with 0.604 gm of each substance : Experimental

sample, 32.26 scale divisions per min (calculated for complete exchange, 32.43 scale divisions per min); standard sample, 64.86 scale divisions per min.

In a further experiment the standard substance used was the residue obtained by evaporating the mother liquor from the precipitated formate. The accuracy of the experiment was sufficient to establish the uniform distribution of the activity between formate and acetate. In this experiment 1.00 gm of formate and 1.30 gm of acetate were dissolved in 20 ml of water.

Measurement with 0.134 gm of each substance: Experimental substance, 22.38 scale divisions per min; standard 20.61 scale divisions per min.

3. Plumbous Acetate (Active) and Plumbic Acetate in Glacial Acetic Acid

Plumbous acetate (1.60 gm) was dissolved in a little glacial acetic acid and the whole was poured into 50 ml of hot glacial acetic acid containing 2.20 gm of dissolved crystalline plumbic acetate[1]. The solution, after clarifying by filtration, was kept for 10 min at 80°C, diluted with water to four times the volume and boiled. Measurements were made on the deposited lead peroxide after washing with dilute acetic acid and alcohol and drying. The residue obtained by evaporating an aliquot part of the filtrate was used as standard.

Measurements were made with 0.136 gm of each substance: Experimental sample, 20.00 scale divisions per min; standard, 21.95 scale divisions per min.

4. Plumbic Acetate (Active) and Plumbous Acetate in Glacial Acetic Acid

The active plumbic acetate was prepared by the addition of an active sample of red lead, obtained by the oxidation of lead monoxide[2], to glacial acetic acid. A portion of the beautifully crystalline acetate was used as a standard. Plumbic acetate (1.72 gm) and 1.48 gm of plumbous acetate (Kahlbaum) were dissolved in 15 gm of hot glacial acetic acid to an almost completely clear solution. The plumbic salt which crystallized on cooling the solution for some time was washed with cold glacial acetic acid, and dried by pressing and in a partial vacuum.

Measurements on 0.400 gm of each substance: Experimental sample, 1.29 scale divisions per min (calculated for the case of complete exchange, 1.43 scale divisions per min); standard, 2.86 scale divisions per min.

[1] A. Hutchinson and W. Pollard, *Soc.* **63,** 1136 (1893); *Ibid.* **69,** 212 (1896). See also A. Colson, *C. R. Acad. Sci, Paris* **136,** 676, 891, 1666 (1903).

[2] L. Vanino, *Präparative Chemie* **1,** 488 (1913).

5. Lead Chloride (Active) and Tetraphenyl Lead in Pyridine

Tetraphenyl lead, $Pb(C_6H_5)_4$, can be prepared in accordance with the description by P. PFEIFFER and P. TRUSKIER[1]. The compound can be recrystallized from hot pyridine or amyl alcohol.

Tetraphenyl lead (1.70 gm) and then 0.92 gm of lead chloride were dissolved in 95 ml of pyridine at the temperature of the boiling water bath and, after heating for ¼ hr, the solution was cooled to about 35° C. A mixture of the two substances crystallized from which the lead chloride was extracted by boiling with water. The lead chloride showed the same activity as before the experiment.

Measurement with 0.180 gm of each substance: Lead chloride before the experiment, 7.39 scale divisions per min; lead chloride after the experiment, 7.32 scale divisions per min.

6. Plumbous Acetate (Active) and Tetraphenyl Lead in Amyl Alcohol

Some acetate (0.70 gm) and 1.00 gm of the tetraphenyl compound were dissolved in 70 ml of hot amyl alcohol and the solution was kept near its boiling point for 15 min. Crystallization of tetraphenyl lead took place gradually on cooling and became complete overnight. The filtered product was thoroughly washed with amyl alcohol, ethyl alcohol and hot water, in succession, and dried in a vacuum desiccator. This sample proved to be completely inactive whereas a sample of the lead acetate prepared as a standard was very radioactive.

Measurement with 0.800 gm of each substance: Tetraphenyl lead after the experiment, less than 0.02 scale divisions per min; lead acetate (standard substance), 180.0 scale divisions per min.

7. Lead Nitrate (Active) and Diphenyl Lead Nitrate in Dilute Ethyl Alcohol

Diphenyl lead nitrate crystallizing with two molecules of water, viz. $(C_6H_5)_2Pb(NO_3)_2 + 2\ H_2O$, was prepared by the method of A. POLIS[2] by adding tetraphenyl lead to nitric acid.

[1] P. PFEIFFER and P. TRUSKIER, *Ber. dtsch. chem. Ges.* **37,** 1125 (1904); cf. K. A. HOFFMANN and V. WÖLFL, *Ibid.* **40,** 2428 (1907).

[2] A. POLIS, B. **20,** 717 (1887). This information was confirmed by P. PFEIFFER and P. TRUSKIER, *Ber. dtsch. chem. Ges.* **37,** 1125 (1904). — The observation already made by POLIS that the formation of diphenyl lead nitrate is disturbed by the appearance of dark coloured substances when the hot concentrated nitric acid is cooled a little below its boiling point is an interesting one. Only the boiling acid can therefore effect the smooth course of all intermediate steps since, otherwise, the reaction takes a different course.

A weight of 1.00 gm of lead nitrate and 1.59 gm of the diphenyl compound were dissolved in 30 ml of 48 % hot alcohol in the presence of two drops of dilute nitric acid. Since there was no deposit within ½ hr, the solution was evaporated almost to dryness on a water bath and the crystalline mixture taken up in 30 ml of 95 % hot alcohol, lead nitrate then being deposited on controlled cooling. This was treated six times with boiling absolute alcohol and dried in a vacuum. A sample of the original lead nitrate served as the standard sample.

Measurement with 0.355 gm of each substance : Lead nitrate before the experiment, 5.55 scale divisions per min ; lead nitrate after the experiment, 5.80 scale divisions per min.

Summary

(1) It has been found that organically bound lead atoms do not undergo intermolecular place exchange in a homogeneous phase.

(2) Such place exchange occurs to an extent corresponding to that calculated from probability when the lead atoms are dissociable.

(3) Radioactive indicator methods were employed to obtain these results.

Comment on papers 7—9

In paper 7 it was shown that a kinetic interchange takes place between the lead atoms of solid lead chloride and the lead ions of a surrounding saturated lead chloride solution. This problem was later studied in detail by Paneth. He found that the uppermost molecular layer of lead sulphate powder participates only in an interchange process. When investigating the behaviour of natural crystals of lead compounds in several cases he found just a fraction of the lead atoms of the uppermost molecular layer participated in a kinetic interchange, presumably positioned at the edges of the crystal. The other extreme case, an interchange of almost all atoms of a precipitate with those of the surrounding solution, was observed in the case of freshly prepared silver bromide by Langer and by Zimmer.

In paper 7 the velocity of dissolution and that of interchange of massive and molecular layers was compared; among other things, it was demonstrated that the velocity of dissolution of ThB is diminished in the presence of lead ions in the surrounding solution; however, the velocity of dissolution of the bismuth isotope ThC was not diminished. This investigation, carried out between 1913 and 1914, aimed at the demonstration of the identity of the behaviour of isotopes. When extending these studies to an interchange between the lead atoms of a lead foil and the surrounding lead ions, several hundred atomic layers were found to be involved in an interchange process presumably due to a dissolution of more electropositive parts of the lead foil followed by a precipitation of lead atoms on more electronegative parts of the foil. An adsorption of lead ions on the metallic surface takes place as well, but its role is insignificant compared with the interchange of lead atoms. An investigation of the behaviour of colloidal lead particles carried out by the author and M. Biltz in 1929 brought out a marked adsorption of lead ions by the colloidal lead particles and a slow interchange only between the lead atoms of the colloidal particles and the lead ions of the liquid phase. In a simultaneous investigation of a system composed of colloidal copper and silver ions (i.e. two metals showing a marked difference in their electrochemical potential) besides some adsorption of silver ions on copper colloids an intense replacement of copper atoms by silver atoms was observed.

In papers 8 and 9 interchange of atoms between heterogenous phases was studied. Paper 9 contains a report on experiment aimed at the elucidation if and to what extent interchange of atoms takes place in a homogeneous phase. When dissolving in the same solute non-radioactive tetraphenyl lead and labelled lead chloride, or vice versa, no interchange of lead atoms was observed; this is in contrast to a solution containing 1 mole of non-radioactive lead nitrate and 1 mole of labelled lead chloride; after subsequent separation by crystallization an equipartition of the radioactive lead atoms was found to take place between the chloride and nitrate of lead. The last mentioned result can be considered to be the most direct proof of the theory of electrolytic dissociation.

Reference

A. Langer (1943) *J Chem. Phys.* **11**, 11
K. Zimmer (1946) *Arkiv f. Kemi*, A 21, No 17.
G. Hevesy and M. Biltz (1929) *Z. Phys. Chem.* B **3**, 271.

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10. SELF-DIFFUSION IN SOLID LEAD

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We have recently shewn[1] that the velocity of self-diffusion, that is, the velocity with which the atoms (molecules) of molten lead change places, can be ascertained by determining the velocity with which a radioactive lead isotope spreads in molten lead. Experiments will now be discussed whose purpose is the determination of the self-diffusion velocity in solid lead.

The extraordinarily high resistances which oppose place exchange in the solid state led from the outset to the expectation of very slow self-diffusion in solid lead ; we have, therefore, avoided setting up experiments at room temperature and have sought rather to determine the self-diffusion in lead heated and maintained about 40°C below its melting point.

Several series of experiments lasting from 1 to 3 months showed that the self-diffusion velocity of lead at 280°C, that is, 46° below its melting point, is less than 0.001 cm²/day. A series of experiments was then performed in which lead filaments, about 2 cm long, were heated for more than 400 days ; these filaments consisted, as will be described in detail below, of a 1.5 cm long inactive and a 0.5 cm long active portion of lead. No diffusion of the active lead isotope into the inactive lead could be detected even after this long period of experiment. The self-diffusion constant of the solid lead is accordingly still smaller than 0.0001 cm²/day, even at a temperature of 280°, since values of this order could still have been easily determined in the stated conditions.

This result is not without interest, especially when it is compared with the well-known Roberts-Austen experiments[2]. Roberts-Austen allowed gold to diffuse into solid lead and found the diffusion constants recorded in the table below, which also includes our experimental result.

Even at 251°C, therefore, the diffusion of gold into lead is at least three thousand times as fast as that of lead into lead at 280°C, the

[1] J. Groh and G. Hevesy, *Ann. Phys.* **63,** 85 (1920).
[2] W. C. Roberts-Austen, *Phil. Trans.* **187,** 404 (1896).

T (°C)	Gold in lead D (cm^2/day)	Lead in lead D (cm^2/day)
100	0.00002	—
165	0.0045	—
200	0.0075	—
251	0.026	—
280	—	< 0.0001

latter temperature being somewhat higher and thus more favourable to diffusion. At this latter temperature, which is not very far removed from the melting point, the self-diffusion is still extraordinarily slow and should be incomparably slower, for example, at room temperature. When relating this result to the velocity of self-diffusion in other metals it must be borne in mind that lead is one of the softest metals and that self-diffusion should presumably prove to be much slower in the harder metals.

Quantitative data on the diffusion in solid metals have been provided only by Roberts-Austen, but metallurgy is plentifully supplied with qualitative experiences which point out the relatively rapid diffusion of alloying solid materials, of which the rapid interpenetration of iron and carbon[1] supplies at 250°C the best-known example. Thus there exists a very considerable difference between the diffusion of two solid metals into each other and the self-diffusion in a solid metal, in complete contrast to the diffusion in the liquid media. Thus, we have obtained[2] a value for the self-diffusion constant of molten lead which is only slightly different from the constant for gold in lead.

The main reason why self-diffusion in lead is so much slower than the diffusion of gold into lead appears to be that the gold diffusing into the lead loosens up the crystal structure and in this way facilitates its own transmission. It is found that the introduction of an impurity into the crystalline structure can have exactly the same effect as a rise of temperature in facilitating the place exchange of the ions (atoms, molecules).

However, it is not stated absolutely that all other metals diffuse more easily into lead than do its own atoms ; we attempted to allow simultaneous diffusion of the lead isotope radium-D and polonium (which is a homologue of tellurium) into lead, but no positive result was obtained.

Diffusion experiments in solid bodies claim so much interest, for this and other reasons, because information can be obtained from the results concerning the magnitude of the resistance opposing the displacement of individual atoms in the crystal structure. Diffusion experiments of

[1] M. A. Colson, *Ann. Chim. et Phys.* **17,** 221 (1846).
[2] J. Groh and G. Hevesy, *Ann. Phys.* **63,** 85 (1920).

the ROBERTS-AUSTEN type, however, are not suitable for obtaining the desired information on this point. If it were required to decide upon the slowness of place exchange in solid lead from the ROBERTS-AUSTEN data a completely erroneous result would be given, whereas the application of radioactive indicators, i.e. the measurement of the velocity of diffusion of a lead isotope in lead yields the required information.

EXPERIMENTAL METHOD

The layers of active and inactive lead were joined together by the method already described[1]. The inactive lead was melted in a vacuum in one limb of the Y-shaped hard glass tube and, after it had solidified, the fused active lead contained in the other limb was poured on, thereby producing a cohesive metallic cylinder. While the active material used in the determination of the velocity of self-diffusion in molten lead was ordinary lead labelled with ThB, this procedure was no longer admissible in the present instance because the ThB decays with a half-life of 10.6 hr and the time of experiment amounted to more than 1 year. Joachimsthal lead, a mixture of ordinary lead, uranium-lead and RaD, has therefore been chosen as the active material. Of these three lead isotopes only the RaD is active and this only to such a small extent that its radiation is not suitable for determining the amount of RaD present; the α-rays of its daughter product, polonium, however, serve as a convenient means for determining the RaD.

Another point in which the experimental method followed here differed from that used for diffusion in the liquid was that, after joining together the layers of active and inactive lead, the boundary surfaces were fused together by means of a finely pointed flame in order to obtain complete contact between the two kinds of lead, this being clearly of great importance for uninterrupted diffusion. A completely cohesive column was thus obtained but of course mixing of the sharp boundaries of the active and inactive lead was unavoidable. In order to take account of this fact, we proceeded as follows :

The column of lead, moulded in the manner described, was cut into two vertical sections with a toothed saw and one of these strips was sealed in an evacuated glass tube which was then placed into an electrical resistance furnace. After the experiment the strip was sectioned at three places marked with India ink and was thus separated into four equal parts. The second vertical strip had already been cut at the corresponding places before the experiment and was used as a control. If the active layer of lead is denoted by I, then layer II likewise showed some activity

[1] J. GROH and G. HEVESY, *Ann. Phys.* **63**, 85 (1920).

on account of the mixing at the boundary; this activity, found even before the diffusion, could, however, be subtracted from that found after diffusion and the mixing at the boundary could be taken into account in this way. Yet the correction described would only acquire importance in the case of an experiment giving a positive result; since no activity noticeably in excess of the natural decay was found in layers III and IV, it was unimportant.

In order to be able to measure accurately the α-activity of the individual sections we have also used here, as in the experiments which served for determining the self-diffusion in molten lead, rolled sheets of lead and measured the activity of the disk thus obtained in the α-electroscope. The total length of filament amounted to 16—20 mm.

The α-activity of polonium indicated by the electroscope is a measure of the amount of the lead isotope (RaD) present only if the RaD and Po exist in radioactive equilibrium. The amount of RaD which diffused in the first four months had come to more than 80 per cent radioactive equilibrium when 14 months had elapsed; that which diffused in the second 4 months had reached over 50 per cent after the same time, when the measurements were made. The absence of any appreciable activity in the layers III and IV made it possible to determine the diffusion constants of both lead in lead and polonium in lead at 280°C as being less than 0.0001 cm²/day, without awaiting exactly the establishment of the radioactive equilibrium between RaD and Po. It is intended, however, to follow this process further during the next year and thus to be able to extend the observations beyond the determined limits of the diffusion constants mentioned above. We are also concerned in working out other types of method which permit the determination of very much smaller diffusion constants than those mentioned.

We may mention here that twelve lead filaments have been prepared as described above, and have been introduced separately into evacuated glass tubes and heated in an electric resistance furnace for 400 days. The furnace temperature, which varied between 270 and 290°C, was followed constantly with a quartz thermometer, since our experience with continuously heated glass thermometers in similar experiments has been unsatisfactory.

Summary

The self-diffusion velocity of solid lead has been determined by following the diffusion of the lead isotope radium-D in solid lead at 280° C for more than 1 year. The diffusion constant, even at this temperature which is only 46° below the melting point, is shown to be still less than 0.0001 cm²/day. Self-diffusion in lead thus takes place at least two-hundred times more slowly than the diffusion of gold in solid lead at the same temperature.

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11. SELF-DIFFUSION IN SOLID METALS

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The "sagacity" with which atoms, or groups of atoms, oscillating about fixed points in the crystal lattice, refuse to exchange position with neighbouring atoms, is often regarded as one of the chief characteristics of the crystalline state. On the other hand, numerous cases are recorded in which crystalline bodies, for example, solid metals, penetrate into each other, in which, therefore, a replacement of the atoms of one metal by those of the other takes place. The classical experiments of Roberts-Austen on the diffusion of gold in lead bars are widely known. At a temperature as low as 100° he found the diffusion coefficient of gold in lead to be 2×10^{-5} cm^2 day^{-1}, being thus only about 100,000 times smaller than that of sodium chloride in water. Several cases of interpenetration of solid metals have been recorded since, including the interesting case of the diffusion of thorium in heated tungsten wires, reported recently by Langmuir. But it must be noticed that from the rate at which one metal like gold diffuses in another like lead, no conclusion can be drawn about the velocity with which the atoms change their position either in a bar of pure lead or of pure gold ; no conclusion can be drawn on the rate of self-diffusion in these elements.

The idea of self-diffusion was introduced by Maxwell, when calculating the rate of diffusion of gases. The calculation was very much simplified by considering the case in which the molecules of the two diffusing gases had the same properties, for example, the exchange of place of molecules in a column of nitrogen. The use of the radioactive isotopes of lead enabled one of the writers, in collaboration with J. Groh (*Ann. d. Phys.* **65**, 216 [1921]) to realise a measurement of self-diffusion in the case of liquid and solid lead, the diffusion in liquids and solids being practically independent of the difference in the masses of the isotopes. For the rate of the self-diffusion in molten lead, namely, of thorium B in molten lead, close to the melting point, the value found was 2 cm^2 day^{-1}. In the solid metal, however, after heating a bar, the upper part of which was composed of radio-lead, for about a year at 280°, and then analysing the lower part with the electroscope, no diffusion could

be found. It was, therefore, concluded that the self-diffusion in solid lead is, even at this high temperature, less than 10^{-4} cm^2 day^{-1}.

To increase the sensitiveness of the method, we prepared in the present work two thin foils, one of ordinary lead, the other with lead containing thorium B in homogeneous mixture, and pressed these together *in vacuo*. The thickness of the inactive foil was chosen slightly greater than the range of the α-particles to be measured; therefore no scintillations originating from the radioactive lead could be observed when investigating the inactive foil. But, on heating the aggregate of the foils, a diffusion of the active lead into the inactive one took place and the α-particles due to the diffused atoms or their successive products of disintegration produced scintillations on the observing screen. By comparing the number of these scintillations with the number of scintillations produced by the active foil at the beginning of the experiment, the rate of self-diffusion in lead was determined. The following values were found:

$t°$	D in cm^2 day^{-1}	$t°$	D in cm^2 day^{-1}
260°	6×10^{-7}	310°	5.7×10^{-6}
280°	1.5×10^{-6}	320°	4.7×10^{-5}
300°	2.5×10^{-6}	324°	1.4×10^{-4}

The diffusion rate 2° below the melting point is thus 10,000 times smaller than in molten lead.

When investigating the diffusion of two very similar metals like silver and gold, or thallium and lead, into each other, we can expect to find conditions not very far removed from those encountered in the case of self-diffusion. By using a foil of thallium and one of active lead it was found that the coefficient of diffusion of lead in thallium amounts at 285°, *i.e.* 15° under the melting point of the latter, to 2×10^{-5} cm^2 day^{-1}.

On the other hand, when investigating the diffusion of two not similar metals into each other, much more intricate conditions were to be expected. We determined the rate of diffusion of polonium, which is the highest homologue of sulphur, into both lead foils and single crystals. The coefficient was found about the same both in the foil and crystal (at 310° $D = 1.3 \times 10^{-5}$ cm^2 day^{-1}). In this connexion it may be mentioned that, in discussing the discrepancy between the values of the period of decay of polonium found by different investigators, Mme. CURIE has put forward the explanation, that during the long time of observation, the polonium in some cases diffused into the metal from the surface of which it was collected. Recently, MARACINEANU (*C. R.* **176.** 1879, (1923)), working in Mme. CURIE's laboratory, has obtained evidence that the apparent period of polonium is appreciably shorter if the lead on which it is collected is heated for a while.

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12. THE HEAT OF RELAXATION OF THE LEAD LATTICE

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The heat of relaxation of the lead lattice (the work of release of the lead atoms) is determined from the temperature coefficient of the velocity of diffusion of a radioactive lead isotope in lead; a study is made of the sensitivity to structure of this quantity and the velocity of diffusion.

Besides the heat of vaporization, heat of fusion and lattice energy there is another quantity of energy which is important to the crystalline state of aggregation. This is the heat of relaxation of the crystal lattice, or the energy of release of the lattice components. The latter is the energy of activation of self-reaction, which takes place between the atoms of a metal and results in place exchange of the lattice units. When the transport number is known the heat of relaxation of ions of an electrolytic conductor can be calculated from the temperature coefficient of the conductivity. Direct measurement of the velocity of self-diffusion is the only course open in dealing with metals. The heat of relaxation, Q, is calculated from the diffusion constant, D, measured at various temperatures, by using the well-known formula

$$D = Ae^{-Q/RT}$$

where A is a constant which is practically independent of the temperature. In the measurement of self-diffusion it is usually necessary to follow the speed of mixing of two closely related metals, such as gold and silver or tungsten and molybdenum, which is then equated as a first rough approximation to the speed of self-mixing of one component. The velocity of self-diffusion in lead can be determined accurately, without such an uncertainty, by measuring the velocity of diffusion of a radioactive lead isotope in ordinary lead. Previous experiments of this kind have already been described[1].

This paper will deal with the result of an investigation carried out recently with the object of determining the heat of relaxation of the lead lattice and of ascertaining how far this quantity and the velocity

[1] G. Hevesy and A. Obrutschewa, *Nature* **115**, 674 (1925).

of self-diffusion in lead are structure sensitive. The method used has been described in detail on a previous occasion[1]. The lead isotope ThB is condensed on a lead surface and the ionization caused by the α-radiation of the radioisotope (or its decay products) is measured before and after the course of diffusion. The deeper the thorium-B penetrates by diffusion into the lead, the greater is the absorption of the α-radiation and the smaller will be the ionization arising from it. The calculation of the velocity of diffusion of thorium-B in lead, which is the same as the velocity of self-diffusion of lead, is executed by means of a formula developed by R. FÜRTH[2], which correlates the ionization values before and after diffusion, the range of the α-radiation in lead and the time. In a more sensitive modification of the method which has also been described the recoil yield, instead of the ionization due to the α-radiation, is measured before and after diffusion.

Both "Kahlbaum" lead and lead of very high purity, which was kindly made available by the Akkumulatoren-Fabrik A. G., Hagen, Westphalia, were used in the experiments. The lead was freed from its content of gases by prolonged fusion in a vacuum and was purified from oxide content by passing it through a capillary system. The lead single crystals were prepared by the method of KYROPOULOS and were characterized, as compared with crystalline lead, by their remarkable resistance to oxidation by the air. All the experiments described below were carried out with complete exclusion of air either in an atmosphere of nitrogen or in a vacuum, and the small tubes containing the single crystals were broken in the evacuated apparatus. All the results recorded in Table 1 can be represented by the equation:

$$\text{or } D = 5.76 \times 10^5 e^{-14025/T}$$

$$\log D = 5.76 - 04343\,(14025/T)$$

or by a straight line (Fig. 1). Thus Q amounts to 14025 $R = 27870$ cal/mole and $A = 5.76 \times 10^5$ and, within the limits of experimental error, there is no difference between the behaviour of the single crystals and the crystalline material.

An investigation was then made as to whether destruction of the texture at the surface of the single crystal by a milling machine, with the specimen necessarily being exposed to the air for a short time, has a measurable effect on the self-diffusion constant. No marked effect on the velocity of diffusion due to this manipulation could be found. Table 2 shows the results of these measurements. The values thus obtained can also be represented by the above equation and by the straight line applying to the unworked material.

[1] G. HEVESY and W. SEITH, *Z. Phys.* **56,** 790 (1929); *Ibid.* **57,** 869 (1929).
[2] R. FÜRTH, *Handb. d. phys. u. techn. Mechanik* **7,** 687 (1930).

TABLE 1. — SELF-DIFFUSION IN LEAD SINGLE CRYSTALS DIFFUSION CONSTANT (D) OF ThB IN LEAD (SELF-DIFFUSION CONSTANT OF LEAD)

No.	t (°C)	D (cm²/day)	$(1/T)10^6$	log D	Remarks[1]
1	182	$4.12 \cdot 10^{-8}$	2197	—7.39	
2	196	$5.7 \cdot 10^{-8}$	2131	—7.24	
3	207	$8.2 \cdot 10^{-8}$	2083	—7.09	Crystalline lead
4	222	$2.45 \cdot 10^{-7}$	2020	—6.6	Single crystal from
5	238	$7.3 \cdot 10^{-7}$	1956	—6.1	the melt
6	245	$9.6 \cdot 10^{-7}$	1930	—6.0	
7	245	$6.57 \cdot 10^{-7}$	1930	—6.18	Single crystal from the melt
8	258	$1.1 \cdot 10^{-6}$	1883	—5.96	
9	259	$3.4 \cdot 10^{-6}$	1879	—5.47	
10	263	$2.3 \cdot 10^{-6}$	1865	—5.64	
11	275	$6.0 \cdot 10^{-6}$	1824	—5.22	
12	290	$7.2 \cdot 10^{-6}$	1776	—5.15	
13	301	$1.6 \cdot 10^{-5}$	1742	—4.79	
14	312	$1.62 \cdot 10^{-5}$	1709	—4.79	
15	317	$2.82 \cdot 10^{-5}$	1695	—4.55	Single crystal from the melt
16	322	$2.36 \cdot 10^{-5}$	1680	—4.63	Crystalline lead
17	324	$4.78 \cdot 10^{-5}$	1674	—4.32	

[1] In all cases where there is no remark single crystals grown by the Kyropoulos method were used; in items 6, 8 and 13 these consisted of Kahlbaum lead and in all other items of lead from the Akkumulatorfabrik, Hagen, Westphalia.

TABLE 2. — DIFFUSION CONSTANT OF ThB IN COLD-WORKED LEAD

No.	(°C)	D (cm²/day)	$(1/T)10^6$	log D	Remarks
18	196	$4.13 \cdot 10^{-8}$	2130	—7.38	Milled single crystal
19	217	$2.87 \cdot 10^{-7}$	2040	—6.54	Milled single
20	233	$4.57 \cdot 10^{-7}$	1976	—6.34	Milled crystallite
21	237	$6.75 \cdot 10^{-7}$	1960	—6.17	Milled single crystal
22	254	$2.99 \cdot 10^{-6}$	1897	—5.64	Milled crystallite
23	270	$4.57 \cdot 10^{-6}$	1841	—5.34	Tempered crystallite

The results discussed above relate to a temperature range which extends from the melting point of lead (327°) to 182°. Below the latter temperature the self-diffusion can be followed by making use of the recoil effect. As has already been mentioned, this method does not make use of the decrease in ionization after diffusion but the recoil yield is determined. While the range of this α-radiation in lead is 3×10^{-3} cm the range of the recoil particles extends only to a thickness of about one hundred atom layers (4.7×10^{-6} cm). In corresponding degree to

the shorter range of the recoil particles the latter method is indeed considerably more sensitive than the method first described. In the study of the self-diffusion of lead ions in lead iodide it has been found possible to determine that the measurement of α-radiation and recoil yield the same result ; in spite of the fact that the recoil measurements register processes in the top few hundred molecular layers they were

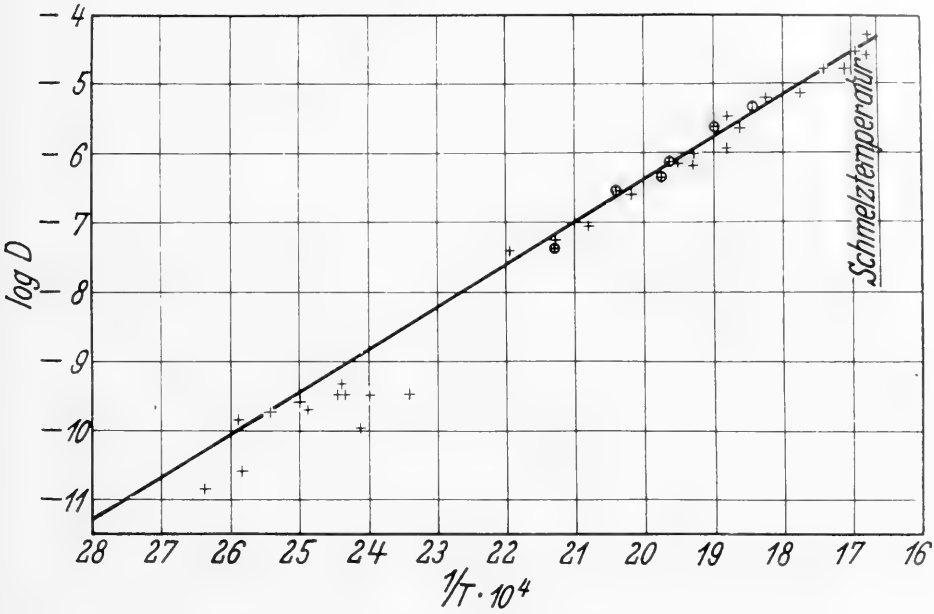

Fig. 1. Rate of Diffusion of labelled solid Lead. Schmelztemperatur—Melting point.

as highly reproducible as the α-measurements. Recoil measurements at lead surfaces, on the contrary, indicated a high sensitivity of the metallic surface to external effects. For example, contact of the lead sample with air for a short time was sufficient to lower the diffusion values and even the values obtained in a carefully purified nitrogen atmosphere were rather lower than those determined when working in a vacuum. In spite of the uncertainty arising for these reasons in the recoil values, the experimental points obtained by this method also lie approximately on the straight lines obtained with the α-measurements as the basis (cf. Fig. 1) ; it must also be borne in mind that the recoil range in lead is not accurately known and that it must be calculated by extrapolation from the values measured in air.

The temperature coefficient of the velocity of diffusion is in quite good agreement with the results of the already mentioned preliminary experiments, but the difference in behaviour of single crystals and crys

TABLE 3. — DIFFUSION CONSTANT (D) OF THORIUM B IN LEAD DETERMINED BY THE RECOIL METHOD

No.	t (°C)	D (cm²/day)	$(1/T)10^6$	log D	Remarks on heating of the lead
1	106	$1.45 \cdot 10^{-11}$	2640	10.84	Nitrogen
2	113	$1.42 \cdot 10^{-11}$	2590	9.85	Vacuum
3	114	$2.60 \cdot 10^{-11}$	2584	—10.59	Nitrogen
4	120	$1.93 \cdot 10^{-10}$	2544	— 9.72	Vacuum
5	128	$2.35 \cdot 10^{-10}$	2494	— 9.63	Vacuum
6	129	$1.97 \cdot 10^{-10}$	2487	— 9.71	Vacuum
7	136	$3.41 \cdot 10^{-10}$	2444	— 9.47	Nitrogen
8	137	$4.59 \cdot 10^{-10}$	2438	— 9.34	Vacuum
9	137	$3.41 \cdot 10^{-10}$	2438	— 9.47	Nitrogen
10	141	$1.08 \cdot 10^{-10}$	2415	— 9.97	Vacuum
11	144	$3.21 \cdot 10^{-10}$	2397	— 9.49	Vacuum
12	153	$3.29 \cdot 10^{-10}$	2346	— 9.48	Nitrogen

tallites demonstrated in those experiments could not be reproduced, possibly because the single crystals in the preliminary experiments were unavoidably exposed for a long time to contact with the air (during counting of the scintillations). The present study shows much more pointedly that neither the heat of relaxation of the lead lattice nor the velocity of self-diffusion of lead atoms is structure-sensitive. This result is possibly connected with the ready recrystallizability of lead since in the molybdenum—tungsten system VAN LIEMPT[1] was unable to find a structure dependence of Q, yet A and therefore the diffusion constant showed such dependence. He found A to be about eight times as large in polycrystalline material as in a single crystal, by measuring the velocity of diffusion of molybdenum in tungsten, and even earlier a structure sensitivity of the electrolytic conductivity, which is closely related to the self-diffusion, had been demonstrated in salts[2].

VAN LIEMPT calculates the constant A from the equation

$$A = \pi x^2 \nu/6$$

where x is the distance between lattice planes and ν the vibrational frequency of the atom. In the case of molybdenum diffusing in tungsten single crystals he finds remarkably good agreement between the observed and calculated values of A. The value of A which we have measured in lead is, on the contrary, about one thousand times the value calculated

[1] S. A. M. VAN LIEMPT, *Z. anorg. Chem.* **195,** 366 (1931); *Rec. Trav. Chim.* **51,** 114 (1932).

[2] G. HEVESY, *Z. Phys.* **10,** 80 (1922); G. TAMMANN and G. VESZI, *Z. anorg. Chem.* **150,** 355 (1926); T. E. PHIPPS, W. D. LANSING and T. G. COOKE, *J. Amer. Chem. Soc.* **48,** 112 (1926), etc.

by VAN LIEMPT's method. The self-diffusion (self-reaction) in lead accordingly represents, at least formally, an example of a chain reaction in which the chain length is independent of whether single crystals or polycrystalline materials are involved.

The magnitude of the heat of relaxation is compared, in Table 4, with the energy content and the heats of fusion and vaporization.

TABLE 4. — HEAT PROPERTIES OF LEAD

	kcal/g atom
Heat of fusion	1.1
Energy content at the melting point	3.5
Heat of relaxation	27.9
Heat of vaporization	36.2

Table 4 shows clearly that the heat of relaxation is not very much less than the heat of vaporization but that it is very much greater than the energy content at the melting point and the heat of fusion.

Summary

The heat of relaxation of the lead lattice (heat of activation for the self-reaction of the lead atoms) amounts to 27,830 cal/mole. This quantity, like the constant A of the diffusion equation, is only slightly structure sensitive.

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13. DIFFUSION IN METALS

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Diffusion in salt-like compounds is facilitated by means of a relaxation process which occurs when the crystal is heated. This relaxation which exhibits some similarity to activation in the theory of reaction velocity depends chiefly on the size, valency, electron affinity and polarization properties of the lattice components. In silver iodide, for example, where the small univalent strongly-polarizing silver ion contrasts with the large iodide ion which has slight attraction for electrons and is easily polarizable, there is easy detachment of the silver ion and it is well-known that mobilities indeed exceed those occurring in aqueous solutions. In the pure metal the behaviour is altogether different. In such case there is only one kind of lattice component, a high co-ordination number and a high symmetry of charge distribution. Large diffusion velocities cannot therefore be expected in pure metals. Metal alloys are different. In the lead-gold system, for example, the small gold atom which has a high affinity for the valence electron contrasts with the larger lead atom which has a lower electron affinity, and hence there occurs a system which is readily subject to relaxation in which the gold atom easily vacates its position. Roberts-Austen in his classical investigations has already been able to demonstrate that gold diffuses into lead even at moderate temperatures with a considerable velocity. The velocity of diffusion of gold in lead is attained through the speed of dissolution of gold atoms in the gold-lead phase and in its taking up a new position. The gold penetrates into lead but, on the contrary, lead is practically immobile in gold. The diffusion constant for gold in lead is 4×10^{-3} $cm^2\,day^{-1}$ at 150° whereas the diffusion of lead in gold-lead at a temperature of 141° amounts only to 3×10^{-11} $cm^2\,day^{-1}$, i.e. it is smaller by seven orders of magnitude. Lead atoms accommodated near silver atoms are more easily dissolved than those considered above. It has been found that lead diffuses about twice as quickly in silver saturated with lead as in pure lead.

If the gold in lead alloys is replaced by other elements whose properties become more and more similar to those of lead, then these elements show a

steadily decreasing diffusion velocity. The margin between the diffusion velocity of the added element and that of lead thus becomes steadily smaller and the unilateral diffusion becomes gradually less apparent. Considering now an alloy of ordinary and radioactive lead, both constituents will exhibit the same diffusion velocity. This is an example of self-diffusion and therefore a complete mutual replaceability in the

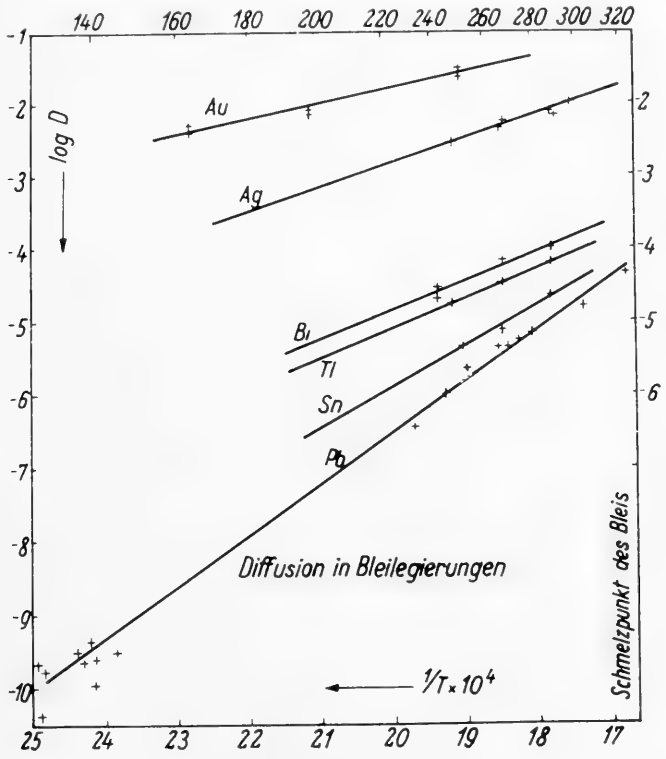

FIG. 1. Diffusion in Bleilegierungen — Diffusion in lead alloys
Schmelzpunkt des Bleis — Melting point of lead

diffusion process. The gradual decrease of diffusion velocities of metals in lead in the sequence, Au—Pb, Pb—Pb, and also the step-wise increase of the heat of relaxation (heat of activation), can be seen in Fig. 1. The latter quantity is also recorded in Table 1.

The marked preferential diffusion of one component is also encountered in salt-like compounds but there is no mutual replaceability of the components which, indeed, is a characteristic of metallic systems. The silver in silver chloride can only change places with silver; it is otherwise in metallic alloys. Considering the place-exchange processes in a saturated silver-lead phase, the readily detachable silver atom will leave its place with great frequency in unit time. The silver atoms intermittently seek out the lead atoms and occasionally also replace other silver

TABLE 1

Metal	D 250° (cm² day⁻¹)	Q (cal)
Au	$3 \cdot 10^{-2}$	~14,000
Ag	$2.6 \cdot 10^{-3}$	15,000
Bi	$3.2 \cdot 10^{-5}$	18,500
Tl	$1.9 \cdot 10^{-5}$	18,500
Sn	$4.4 \cdot 10^{-6}$	*c.* 28,000
Pb	$1.3 \cdot 10^{-6}$	*c.* 30,000

atoms. The lead atoms on the contrary scarcely ever leave their places. At room temperature, a lead atom in pure lead changes its place once in 10 days while the silver atom in lead-silver alloy vacates its position 100 times per sec.[1] As the temperature is raised the difference diminishes. We have already been able to demonstrate, in an earlier paper[2], in the case of silver alloys that the speed of place exchange of the atomsdecreases as the ideal metallic state is approached. The last step, however, i.e. the measurement of self-diffusion in silver, was not practicable in the systems mentioned. We have therefore applied our attention chiefly to lead alloys because they presented an opportunity for determining also the self-diffusion in lead.

In determining the frequently very low velocity of diffusion, use was made of quantitative optical-spectroscopic analysis which has been found especially suitable for this purpose. Indeed this methods permits both the determination of very small amounts of metal and the use of very thin and strictly localized layers in the analysis. Samples of known composition are first prepared, e.g., lead-thallium alloys with the concentration varying between 3 and 0.01 per cent and the ratio of the intensities of suitable lines of the two elements is determined. Layers, each 0.05 mm thick, are cut after diffusion and these are analysed spectroscopically by comparing with the test samples in accordance with the method described by GERLACH[3]. The diffusion constant can be calculated since the concentration, as a function of the distance from the original boundary, is known. In the method described it is above all necessary to ensure that the metal whose diffusion constant is to be determined is distributed as atoms in the lead. If this does not apply then the analytically determined concentration is not identical with

[1] Concerning the calculation of the frequency of place exchange of an individual atom, refer to H. BRAUNE, *Z. phys. Chem.* **110,** 147 (1924); J. FRENKEL, *Z. Phys.* **35,** 652 (1926); J. A. M. VAN LIEMPT, *Z. anorg. Chem.* **195**, 366 (1931).

[2] G. HEVESY, *Z. Elektrochem.* **34.** 463 (1928).

[3] W. GERLACH and E. SCHWEITZER, *Chemical Emission-Spectrum Analysis*, Leipzig (1930). More details of the application of this method to measurements of diffusion will shortly be described by GUENTHER and LAIRD.

the concentration considered in the diffusion process[1]. The silver-lead and gold-lead systems provide examples of the behaviour just referred to. Starting with a concentrated silver-lead alloy, in which the silver is as finely divided as possible, then the silver which has migrated by diffusion will constantly be replaced by dissolution from the grains of silver. The system therefore consists of a constantly saturated solution of silver in lead from which silver diffuses into pure lead. As will be seen later, the solubility of silver in lead can be determined by means of this behaviour.

In only a few cases have we used an alternative to the spectroscopic method. The silver content of lead alloys has been determined, for example, by the usual method of volatilizing the lead and assaying the residual lead of silver. It was also necessary to employ radioactive methods in order to determine the self-diffusion rate. At higher temperatures, where the selfdiffusion is already somewhat larger, the diffusion constant could be determined as follows : A radioactive lead isotope is condensed on the surface of inactive lead and the ionization due to the α-particles emitted by the radioactive lead is measured. The system is then heated to the experimental temperature and the ionization, which now has a lower value owing to the diffusion which has taken place, is measured again. The self-diffusion constant can be determined from this decrease due to diffusion. At a lower temperature this method, which indeed is very sensitive, proved not sensitive enough and had to be replaced by another. The recoil yield before and after diffusion was measured and the diffusion constant was calculated from the decrease by means of a formula developed by R. Fürth[2]. While the first method enables the diffusion constant to be determined down to 10^{-7} cm^2/day, the use of the second method permits diffusion constants of 10^{-13} cm^2/day or less to be obtained.

The solubility of a metal in a solid phase can be determined from the analysis of diffusion since only those particles of the metal which are distributed as atoms are involved in the diffusion process. The discussion is best illustrated by an example.

(a) Starting with a concentrated alloy the diffusion is allowed to proceed until all parts of the originally pure layer of lead are saturated with the diffusing metal. Since an increase in the diffusion time is no longer accompanied by an increase in concentration there now exists a saturated solution the analysis of which yields the solubility directly. At 288°C, for example, it is impossible to produce by diffusion a silver-lead alloy containing more than 0.13 atomic per cent of silver.

[1] cf. G. Grube (*Z. Metallk.* **19,** 438 [1927]), who has determined a series of diffusion velocities in high-melting metals. With regard to the problem of diffusion in alloys refer also to the many publications of G. Tammann and his school.

[2] R. Fürth, *Handbuch der physikal. und techn. Mechanik* **7,** 687 (1930).

This method, of course, requires long experimental times and a time of 3 months is needed for homogenizing a 5 mm layer even with silver which diffuses comparatively rapidly in lead. We have therefore often used the following consideration for determining the solubility:

(b) Disregarding the initial layer (lead-silver alloy) at first, the silver concentration is determined at various positions after diffusion in the originally pure lead and the diffusion constant is calculated from these results. Now if the diffusion constant is known the corresponding concentration of silver can be calculated and this in turn yields the solubility of silver in lead.

Starting with a silver-lead alloy of

10 atomic per cent
1 atomic per cent
0.5 atomic per cent

we obtained the following values for the solubility of silver in lead

0.15 atomic per cent
0.12 atomic per cent
0.13 atomic per cent

Hence, starting with silver-lead alloys of various concentrations, from which the same solubility values have been obtained, it may also be concluded that the silver from the macroscopic grains of silver was supplemented so rapidly that the original lead-silver alloy always remained saturated. If a diffusion experiment is performed, commencing with a 0.1 atomic per cent silver-lead alloy, the same diffusion coefficient found by the method described above is obtained both from the concentration of the silver in the initially pure lead layers and from the concentration decrease of the silver in the original silver-lead alloy. The method described for determining the solubility is important in so far as there are at present no other methods available for determining very low solubilities of one metal in another[1].

Summary

The diffusion of one metal in another solid metal is in most cases an almost unilateral process. For example, the velocity of diffusion of gold in lead is very rapid but lead diffuses extremely slowly in gold. As the two alloying components become increasingly similar, for example, in passing from gold-lead, silver-lead, bismuth-lead, thallium-lead, tin-lead to lead-lead, the one-sided nature of the process gradually disappears. Diffusion measurements make it possible to determine very low solubilities of one metal in another. The solubility of silver in lead at 285°C was thus found to be 0.13 atomic per cent.

[1] In the literature silver is stated to be insoluble in solid lead.

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14. THE APPLICATION OF RADIOACTIVE RECOIL IN DIFFUSION MEASUREMENTS

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A layer of thorium-B chloride placed on the surface of $PbCl_2$ shows a decrease in α-recoil yield after heating. The velocity of diffusion of the thorium-B ion in lead chloride and thus the velocity of self-diffusion of the lead ions can be determined from this effect. This extraordinarily sensitive method by means of which diffusion constants down to 10^{-13} $cm^2\ day^{-1}$ can be determined permits the measurement of the velocity of diffusion in $PbCl_2$ and PbI_2 in the vicinity of 100°C or at a higher temperature.

Two different cases must be distinguished in diffusion in crystalline substances, heterogeneous diffusion and self-diffusion. The difference between these two cases exists also in other states of aggregation but is only slightly perceptible in the liquid and gaseous forms. During heterogeneous diffusion in crystalline substances individual lattice components are replaced by foreign particles or the foreign ions (atoms) intrude into the interstices of the lattice. In self-diffusion the lattice components are replaced by identical particles. Considerable affinities between the diffusing and lattice-element substances often operate during heterogeneous diffusion and we are confronted with a process which is a combination of a chemical reaction, often proceeding with a significant decrease in entropy, and a true diffusion process. Self-diffusion produces merely a positional mixing of the lattice components without any practicable change of entropy. The phenomenon of self-diffusion is employed when information is required on the strength of binding of the individual ions (atoms) in the crystalline compound. In such measurement the method often used is to study the diffusion of an ion, e.g. a cation, in the crystalline compound whose cations are closely related to the diffusing one. For example, the diffusion rate of cuprous ions in silver salts may be measured or that of the cuprous ions in silver salts the cuprous and silver ions being considered to be nearly identical from the standpoint of diffusion. The binding strength of silver and cuprous ions in various compounds can be determined to a fair approximation from such measurements. On the other hand it is not possible to determine the

binding strength of e.g. iodide in silver iodide, by similar measurements, and it must suffice to conclude from TUBANDT's[1] transport measurements and G. G. SCHMIDT's ionic emission experiments that the binding strength of the iodide ion in silver iodide is considerably greater than that of the silver ion. The indication of the last-mentioned result calls to mind another method for determining the velocity of self-diffusion, viz. by calculating from the electrolytic conductivity of the crystalline compound by using the theory propounded by NERNST for electrolytic solutions or by means of the Einstein diffusion equation. This method also, however, yields only the velocity of self-diffusion of the lightly bound ions, that is, silver and cuprous ions in silver and cuprous salts.

The ideal of self-diffusion can be approached extraordinarily closely by allowing a radioactive ion to diffuse in the appropriate compound of an isotopic inactive ion, by applying the method of radioactive indicators, for example, by measuring the diffusion of ThB ions in lead chloride. Now the ions of the radioelements, except those of the thalium isotopes which are too short-lived (half-life always less than 5 min) to be considered, are multivalent. Multivalent ions, however, are always characterized by particularly strong binding[2]. From this it follows that the self-diffusion can be measured by the method sketched out above only with the aid of an extremely sensitive arrangement. The values thus obtained should indeed yield data on the binding strength of even this ion which has practically no share in the electrolytic conductivity and whose velocity of self-diffusion cannot therefore be calculated from conductivity data. With the usual measuring apparatus the procedure of STEFAN is followed by placing several, frequently three, equally thick layers of the diffusion medium on a layer of the diffusing substance. The diffusion constant is inversely proportional to the square of the layer thickness. The smaller the diffusion constant to be measured the less will be the chosen layer thickness. If a velocity of diffusion (D) of, for example, 10^{-8} cm^2 day^{-1} is to be measured then, for an experimental time of 1 day, it is necessary to choose a layer thickness of about 0.01 mm. It is not practicable, however, to place three equally thick inactive layers on a 0.01 mm thick layer of radioactive lead chloride and to separate them again after diffusion for the purpose of radioactive analysis. On the other hand the various radioactive methods yield opportunities to attain such small layer thicknesses in another way. One of the authors with OBRUTSCHEWA[3] has determined the velocity of self-diffusion of lead atoms, both in single crystals and in crystalline lead, by

[1] C. TUBANDT, H. REINHOLD and W. JOST, *Z. phys. Chem.* **29,** 69 (1927).

[2] Compare the transport results of TUBANDT, *Z. phys. Chem.* **29,** 69 (1927); see also E. FRIEDRICH, *Z. Elektrochem.* **32,** 576 (1926).

[3] G. HEVESY and A. OBRUTSCHEWA, *Nature* **115,** 674 (1925).

collecting atoms of the lead istotope on a lead surface and counting the number of scintillations shown by the infinitely thin radioactive coating before and after diffusion in the heated metal. The decrease in the number of scintillations is a measure of the velocity with which the radioactive lead atoms have passed, because of diffusion, outside the range of the α-rays and into the interior of the metal. The layer thickness here required for the diffusion calculation is the range of the α-rays in lead, which amounts to about $^1/_{30}$ mm. Diffusion constants down to 10^{-8} cm^2 day^{-1} were measured with the help of this method. This sensitivity, however, was inadequate in the present study and we therefore used the radioactive recoil effect which can be expected to yield a considerable increase in sensitivity for detection of the diffusion. The range of α-recoil in lead amounts to only 3×10^{-5} mm and thus the application of the recoil method permits measurement of the extraordinary small diffusion constant of 10^{-13} cm^2 day^{-1}. The velocity of diffusion of the ions of the lead isotope ThB (half-life 10.6 hr) was measured in different compounds lead. The recoil yield thus the activity of ThC, was determined before and after diffusion. The radioactivity measurements were made only after the establishment of the equilibrium between ThB and ThC, since the α-rays responsible for the recoil effect are derived not from thorium-B but from its daughter product thorium-C (half-life 1 hr).

EXPERIMENTAL METHOD

The radioactive substance was condensed from the vapour phase on the pellet to be used for measuring the velocity of diffusion. The pellets were prepared by pressing very carefully purified lead chloride or iodide. The pressure used was 1800 kgm/cm^2 and was applied for 1 min.

The pellet was pressed on to the front of a 14 mm diameter brass cylinder and the two together were suspended in the apparatus (Fig. 1). This apparatus consists of two chambers A and B connected by means of a cock with a 2 cm bore. Each chamber can be separately evacuated and filled with purified nitrogen. The brass cylinder with the pellet is fastened by means of a silver wire to a screw, C, situated above the chamber A. A phosphorus pentoxide tube is first attached to the standard joint, S, and the whole apparatus is filled with nitrogen.

The ThB chloride is condensed on to the lead chloride surface in the following way: the active deposit from thorium is collected on a platinum foil and the foil is then exposed to the action of chlorine gas. The foil is transferred into the vaporization apparatus, which is attached at S (see Fig. 1) while the pellet is situated in the chamber A. The vaporization chamber consists of a brass tube K, which can be cooled, into which the brass cylinder with the pellet just fits. The lower joint of

this tube is connected with a matching glass joint, which contains two brass rods for the power supply and across the ends of which the activated platinum foil is fastened horizontally. The chamber *B* and the vaporization chamber are evacuated and filled with nitrogen before the pellet is lowered from *A* until it is near the top of the platinum foil *G*. At a nitrogen pressure of 1 mm the foil is brought to a white heat (about 900°) for 1 sec and thus the vapour of ThB chloride is transferred on to the surface of the pellet.

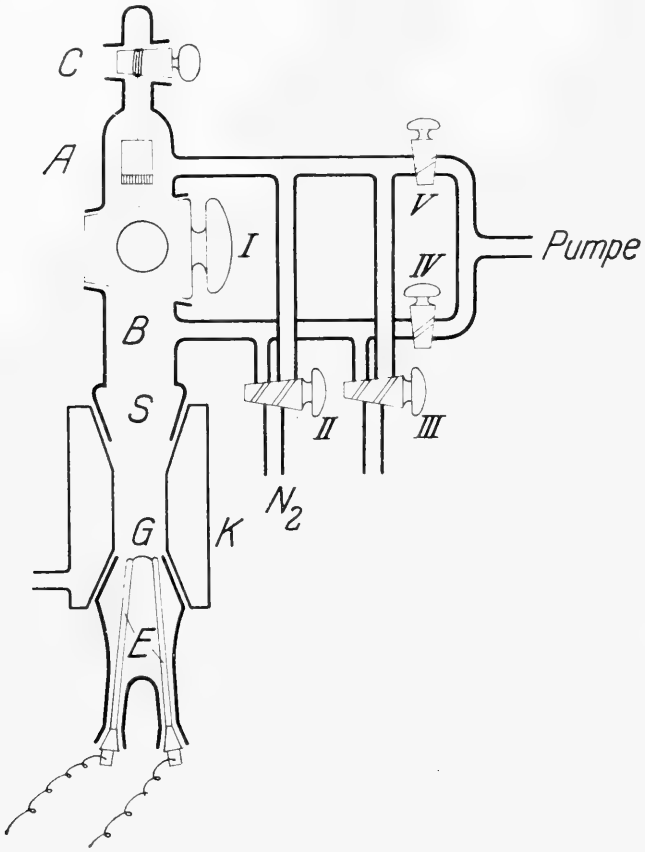

Fig. 1.

The recoil product, ThC″, emits β-rays whose strength constitutes an easily traceable measure of the recoil yield. The recoil product is collected as follows : After first waiting until radioactive equilibrium has been attained the pellet is placed in a tube attached at *S* above a copper foil charged to −220 V, the pellet being earthed, and the recoil atoms are collected on the copper foil. A decrease in the pressure to 2 cm aids the collection of the recoil product. Measurement of the β-activity of the copper foil gives the recoil yield before the diffusion.

The pellet can be brought up to the experimental temperature for a definite time by attaching at S a furnace containing a glass vessel with a standard joint. In the glass vessel is a hollow iron block (1.5 kgm) into which the pellet and the brass cylinder can be introduced such that the direct contact with the metal facilitates rapid equalization of the temperature. The temperature is measured by HOSKINS' method by means of a thermoelement of high thermoelectric power fixed in a side hole in the iron block. This apparatus also is filled with nitrogen.

The lead chloride was prepared from Kahlbaum purest lead chloride by repeated recrystallization from hydrochloric acid solution. It was dried by heating nearly to the melting point in a current of HCl.

The lead iodide was obtained from an active acid solution of HI and $Pb(NO_3)_2$, purified by decantation and dried over P_2O_5. Sublimation was avoided since PbI_2 prepared thus always contains traces of iodine.

CALCULATION

We are indebted to Professor R. FÜRTH of Prague for the formulae used below.

The calculating procedure is as follows: If the recoil activity before the experiment is equal to 1 and after the experiment to A, then $1-A$ atoms of lead have diffused so far in to the pellet that their recoil products are no longer able to leave it. If all the recoil particles moved perpendicular to the surface, then all those issuing from lead atoms which had not diffused deeper than the range, a, of the recoil particles would be able to escape from the surface. The relationship between the number A and the diffusion constant D is therefore

$$A = \int_0^{} \frac{1}{\sqrt{(\pi DZ)}} e^{-x^2/4DZ} \cdot dx \qquad (1)$$

where Z is the time and x is the distance of the particle from the surface. By using the Gaussian error function

$$\psi(\xi) = \frac{2}{\sqrt{\pi}} \int_0^{\xi} e^{-u^2} \cdot du \qquad (2)$$

the equation

$$A = \psi\left(\frac{a}{2\sqrt{DZ}}\right) \qquad (3)$$

is obtained, whence D can be calculated.

It must be borne in mind that in the present instance the recoil particles are expelled in all directions, such that the particle can reach the surface only when the distance x of its starting point from the surface satisfies the condition

$$x/at \leqslant \cos a \tag{4}$$

where a is the angle between the normal to the surface and the ray. The ratio of the number of particles reaching the surface from a point C to the total number of particles issuing from that point in all directions is equal to the ratio of the surface of the spherical cap of height $a-x$ to the surface of a sphere of radius a, whence it follows that

$$\frac{2\pi a(a-x)}{2\pi a^2} = 1 - \frac{x}{a} \tag{5}$$

To take account of this concept the integrand in equation (1) must be multiplied by $(1-x/a)$, and thus

$$A = \int_0^a \frac{1}{\sqrt{(\pi DZ)}} \left(1 - \frac{x}{a}\right) e^{-x^2/4DZ} \cdot dx \tag{6}$$

or, by substituting

$$\xi = a/2\sqrt{(DZ)} \tag{7}$$

the result is

$$A = \psi(\xi) - \frac{1}{\xi\sqrt{\pi}} (1 - e^{-\xi^2}) \tag{8}$$

This equation is evaluated graphically and from the value of ξ thus obtained D is calculated by means of equation (7).

Since the diffusion constant has been calculated in some cases from the decrease in the α-ionization it may be appropriate to discuss the calculation for that method. The α-activity was measured by selecting a parallel beam normal to the pellet surface such that equation (1) could be applied. The shutter had an air gap of 5.3 cm and thus only the α-rays of ThC'', with a range of 8.4 cm, were able to penetrate. The conditions for the calculation were thus simplified. In measuring D by means of α-radiation it must be noted that the particles entering the electroscope do not all have the same ionizing effect since this is dependent on the path already travelled by a particle. A particle which has come from the interior of the pellet has less effect than one which has started from the surface. If the decrease in ionization due to a particle which has travelled a distance x in the PbI_2 pellet in relation to the effect of one which has started from the surface is represented by

$$J = q(x) \tag{9}$$

and if also the retarding effect due to the air column of the shutter is equal to that of a PbI_2 layer with a thickness b, then

$$A = \psi(\xi) - \int_0^{a-b} \frac{1}{\sqrt{(\pi DZ)}} \varphi(x)\, e^{-x^2/4DZ} \cdot dx \qquad (10)$$

In the above equation

$$\xi = (a-b)/2\sqrt{(DZ)} \qquad (11)$$

and the expression is evaluated graphically.

THE DIFFUSION OF LEAD IONS IN LEAD CHLORIDE

If an attempt is made to determine the diffusions of lead ions in lead chloride with the ordinary apparatus of STEFAN, by pressing together a 3 mm deep inactive lead chloride pellet and a 1 mm deep ThB-labelled lead chloride layer, no diffusion of the radiactive lead ions can be detected after 4 days at 480°C. It is not feasible to raise the experimental temperature since the high vapour pressure of lead chloride at the above-mentioned temperature (10^{-1} mm) already causes disturbance. The interference can indeed be partly restrained by carrying out the experiment in a pressure bomb under a nitrogen pressure of 200 atm, but cannot be wholly eliminated. For the reasons mentioned it also seemed hopeless to prolong the duration of the experiment, possibly by replacing ThB by the long-lived RaD. For the same reason, the determination of the diffusion constant of lead chloride, in the vicinity of the melting point, by means of the decrease in α-radiation after heating ThB chloride collected on the surface of a $PbCl_2$ pellet was also a failure. Even at 370° the amount of ThB which evaporates can be detected by radioactive methods. The diffusion constant of lead ions in lead chloride must therefore be measured at lower temperatures at which only the very sensitive recoil method can be considered. The results of the measurements obtained by this method are shown in Fig. 2 and Table 1. The circles relate to a series of experiments in which the active deposit was treated with chlorine. The circles combined with strokes relate to experiments where ThB oxide or sulphide, instead of the chloride, was condensed on the $PbCl_2$ pellet. Another series of results not quoted here yield the same graphical pattern.

The time of experiment was so chosen that the decrease in the recoil yield after diffusion amounted to about 50 per cent. This circumstance is most favourable both for carrying out the experiment and for calculation. It is necessary to know the range of the recoil rays in lead chloride in order to be able to calculate the diffusion constant from equation 8 on

page 130. This quantity is determined as follows : The retarding power of lead and of chlorine for α-radiation is known, whence the range of α-radiation in $PbCl_2$ can be obtained. The ranges of α-radiation and recoil particles in air are known. On the assumption that the ratio of the ranges in air is equal to the ratio in lead chloride, the range of recoil particles in lead chloride calculated from the above data is 7.5×10^{-6} cm.

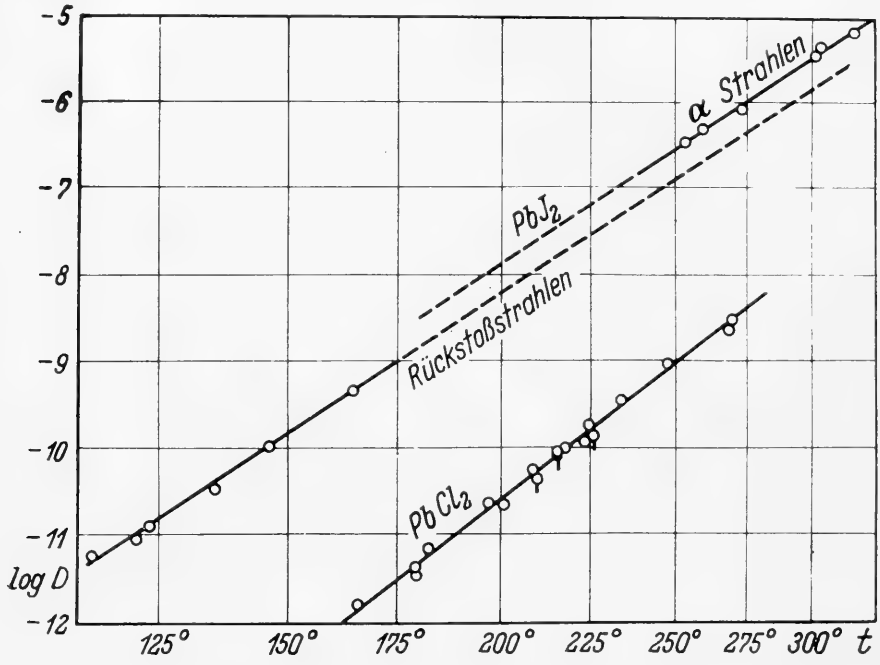

Fig. 2. Self-diffusion of Pb in $PbCl_2$ and PbI_2. α Strahlen = α-radiation Rückstoßstrahlen = recoil radiation

In the discussion of the experiments with PbI_2 a method will be described which permits experimental testing of the correctness of the above value. In order to make sure that vaporization effects have not influenced the results experiments have also been performed at reduced pressure and these have yielded the same results as those at the ordinary pressure.

Attempts to condense ThB oxide or sulphide instead of the chloride were made, in order to obtain information on the effect of a possible incomplete formation of ThB chloride on the experimental results. It is evident from Fig. 1 that the results were not essentially different, owing to the fact that the lead ion surrounded by many chlorine ions soon loses its oxygen partner. The treatment of the $PbCl_2$ pellet with chlorine or HCl, after condensing the active material, is also without effect on the result.

TABLE 1. — SELF-DIFFUSION OF Pb IONS IN $PbCl_2$

t	$1/T$	D	$\log D$	Remarks
166	0.002277	$1.47 \cdot 10^{-12}$	—11.83	
180	2207	4.20	—11.38	
180	2207	4.44	—11.35	
183	2193	6.60	—11.18	
198	2123	$2.38 \cdot 10^{-11}$	—10.62	
201	2108	2.72	—10.57	
210	2070	5.79	—10.24	
211	2068	4.69	—10.33	Sulphide distilled
216	2045	9.68	—10.01	Oxide distilled
217	2040	9.93	—10.00	
220	2028	$1.28 \cdot 10^{-10}$	— 9.89	
223	2018	1.22	— 9.91	
225	2008	1.63	— 9.79	Oxide distilled
225	2008	1.85	— 9.73	
235	1969	3.44	— 9.46	
249	1916	9.00	— 9.05	
268	1847	$2.60 \cdot 10^{-9}$	— 8.58	
270	1841	3.16	— 8.50	

The curve of Fig. 1 can be represented by the equation

$$D = 1.060 \times 10^{7} \quad e^{-38.120/RT}$$

This yields 38,120 cal for the molecular heat of relaxation of lead ions and the value 1.06×10^{-7} for the constant A. The heat of relaxation of the chloride ions in lead chloride is found to be 10,960 cal, from the conductance of $PbCl_2$. The large difference between the heats of relaxation immediately renders intelligible the result of TUBANDT, according to which practically all the mobility in lead chloride is due to the chloride ions. The lead ions require a much greater energy content than the chloride ions to enable them to take part in place exchange processes. The transport number of lead ions in lead chloride is found to be 10^{-5} at 270°C.

The investigation of self-diffusion in single crystalls of lead chloride, in which the present experience suggests a smaller diffusion, will be discussed later.

THE DIFFUSION OF LEAD IONS IN LEAD IODIDE

Lead iodide has a smaller electrolytic conductance than lead chloride ; yet a relatively high diffusion velocity of lead ions in lead iodide would be expected, in spite of the low conductance, since TUBANDT found a high value (0.67) for the transport number of the lead ion in PbI_2. In agreement with this expectation, it is evident from Table 2 that the diffusion constant can be measured even a few degrees above 100°C. It was always necessary to use annealed pellets in order to obtain reproducible values.

We find the molecular heat of relaxation to have a value of 30,000 cal and the constant A to amount to $3.43\ 10^5$.

TABLE 2. — SELF-DIFFUSION OF LEAD IONS IN PbI (RECOIL METHOD)

t	$1/T$	D	log D
114	0.002585	$6.31 \cdot 10^{-12}$	—11.20
122	2532	9.59	—11.02
124	2518	$1.47 \cdot 10^{-11}$	—10.83
137	2440	4.23	—10.37
147	2382	$1.17 \cdot 10^{-10}$	— 9.93
165	2283	6.35	— 9.20

The diffusion constant can be represented by the formula

$$D = 3.43 \times 10^5\ e^{-30.000/RT}$$

Since there is an appreciable mobility of the lead ion in PbI_2 even at temperatures which are very far removed from the melting point it was also possible to apply the decrease of ionization after diffusion, due to the α-particles, for measuring the diffusion constant. The results are shown in Table 3 and Fig. 2. The diffusion constant can be represented by table following equation :

$$D = 9.11 \times 10^5\ e^{-30.140/RT}$$

The heat of relaxation, which amounts to 30,140 cal/mole, does not differ appreciably from the value yielded by the recoil experiments (30,000 cal). This agreement is also expressed in the parallel courses of the curves in Fig. 2. The fact that they do not quite coincide is probably due to some uncertainty attaching to the value of the recoil range, as already mentioned above. The two straight lines could be superposed by assuming the range of the recoil particles in lead iodide to be 0.11 μ instead of 0,075 μ.

Table 3. — Self-Diffusion of Lead Ions in PbI_2 (α-Particle Method)

t	$1/T$	D	$\log D$
255	0.001895	$3.63 \cdot 10^{-7}$	—6.44
260	1876	5.30	—6.28
301	1749	$3.42 \cdot 10^{-8}$	—5.47
302	1739	4.26	—5.37
315	1701	6.70	—5.17

The diffusion constant of lead ions in PbI_2 can also be calculated from the electrolytic conductance of this compound and the transport number. At 390°C, for example, the calculated diffusion constant is 0.9×10^{-6} $cm^2\ day^{-1}$ while the recoil measurement and α-ray measurement yield 0.9×10^{-6} and 2.2×10^{-6}, respectively. The behaviour at low temperatures, where the mobility of the iodide ions controls the conductance, is discussed in the subsequent paper.

Summary

The velocity of diffusion of lead ions in lead chloride and iodide has been measured by making use of radioactive recoil. The values obtained are $D_{PbCl_2} = 1.06 \times 10^7\ e^{-38{,}120/RT}$ and $D_{PbI_2} = 3.43 \times 10^4\ e^{-30{,}000/RT}$. The diffusion velocity values for the lead ion in lead iodide were confirmed by other methods. The high value for the heat of relaxation of lead ions in lead chloride (38,120 cal/mole) explains Tubandt's result, viz. that in lead chloride the chloride ions whose heat of relaxation amounts only to 11,180 cal are practically the only mobile ions. The transport number of the lead ions in lead chloride at 270° is calculated to be 10^{-5}.

The velocity of diffusion of lead ions in lead iodide calculated from the electrolytic conductance and from the transport numbers at 290°C as determined by Tubandt, is in good agreement with our experimental value.

Comment on papers 10—14

When faced with the task of calculating the diffusion rate of gaseous oxygen in gaseous nitrogen, to facilitate the calculation Maxwell made the assumption that the molecules of oxygen and nitrogen have the same radius and same mass; he thus arrived at the notion of self-diffusion. The introduction of the labelling principle made it possible to measure a diffusion rate close to self-diffusion.

In connexion with the discussion of the interchange between lead atoms of a lead foil and the surrounding lead ions the problem of the diffusion rate of solid lead in lead was first raised in 1915 (paper 8). The first experiments in this field described in paper 10 were, however, carried out a three years later only. Prior to this investigation Gróh and the writer measured the rate of diffusion of labelled molten lead in non-labelled lead. Self-diffusion in liquids cannot be expected to lead to results which cannot more or less be foreseen. The rate of diffusion of molten lead in molten lead does not differ much from the diffusion rate of cadmium or thallium in molten lead. In contrast, the rate of self-diffusion in solid metals cannot be foreseen. The diffusion of a solid metal, even a closely related one, in another metal produces changes in the crystalline state which may strongly facilitate penetration. We found that the atoms of solid labelled lead diffused into solid lead about 200 times slower than thallium atoms and about 10,000 times slower than gold atoms diffuse into solid lead. In the first investigation on the diffusion in solids described in paper 9 labelled lead was soldered on a non-radioactive lead rod. After keeping this system at 280°C for up to 400 days slices from the rod close to the place of soldering were prepared and their radioactivity compared. The figures obtained permitted the calculation of the upper limit of the diffusion rate of lead in lead. In a later investigation (paper 11) carried out with Mrs. Obrutsheva, wife of the well-known Russian physical chemist Frumkin, we increased the sensitivity of the method by pressing *in vacuo* a non-radioactive lead foil on one labelled with thorium B. The thickness of the inactive foil was chosen slightly greater than the range of α-particles to be measured; therefore no scintillations originating from the radioactive lead could be observed when investigating the inactive foil. But, on heating the aggregate of the foils, a diffusion of the active lead into the inactive one took place and the α-particles emitted by the succession products of ThB (ThB emits no α-rays, but comes rapidly into exchange equilibrium with α-particles emitting desintegration products) produce scintillations on the observing screen. In further investigations with Seith we replaced the counting of scintillations by ionization measurements. The range of α-particles emitted by the disintegration product of ThB in lead amounts to 3×10^{-3} cm. A replacement within this thickness of some of the ThB atoms by non-radioactive lead atoms due to an interchange process leads to a decrease in the ionization measured. The range of the recoil particles emitted by the ThC, the disintegration product of ThB, is still appreciably (almost 1000 times) shorter than that of the α-particles. The measuring of the decrease of the recoil yield with time of a with ThB covered surface permits to determine as low a diffusion rate as 10^{-13} $cm^2 day^{-1}$. By making use of this method self-diffusion in solid lead taking place at 106°C or at a higher temperature was measured (paper 12). From the change of the rate of self-diffusion with temperature the value prevailing closely to the melting point was calculated

and compared with the rate observed after melting took place. The ratio worked out to be 10,000.

The measurement of the rate of self-diffusion of lead ions in a solid lead salt permitted the determination of the transference number of the ions of solid lead halogenides (paper 14). In his very beautiful investigation TUBANDT found that while both ions have a large part in the conduction of electricity through the solid lead iodide, alone the movement of chloride ions is responsible for the passage of an electric current through solid lead chloride. The ionic mobility of Pb^{2+} in $PbCl_2$ calculated from its self-diffusion rate determined by making use of the very sensitive recoil method indicates that for about $\frac{1}{10{,}000}$ part of the electrical current passing through solid lead chloride the movement of lead ions is responsible.

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15. SEARCH FOR AN INACTIVE ISOTOPE OF THE ELEMENT 84 (POLONIUM)

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The elements 81 (thallium), 82 (lead), and 83 (bismuth) have both radioactive and inactive isotopes, whereas the elements 84—92 are only known in an active form. Several attempts have been made to find inactive isotopes of the latter elements. Aston, using his mass spectrograph, tried to discover a stable isotope of radon in the atmosphere, and Hahn made extensive researches to find an inactive isotope of radium. All these attempts failed.

We have recently tried to extend the series of inactive elements by searching for an inactive isotope of the element 84 (polonium), which follows bismuth. Through the work of the discoverer of this element, Mme. Curie, and her co-workers, as well as of Marckwald and of many others, the chemical properties of polonium were found to be intermediate between those of bismuth and tellurium. Hence it is obvious that if a stable isotope exists, it must be associated in nature with tellurium or bismuth.

We looked for the elements 84, therefore, in the following tellurium and bismuth minerals: Hessite, calaverite, nagyagite, tetradymite, and bismuth glance as well as native bismuth. The minerals were dissolved, and a known amount of polonium added as radioactive indicator. On removal of the polonium from the solution, it was to be assumed that any isotope present in the solution would accompany the active polonium. By special methods devised for the purpose, it was possible to regain the added polonium electrolytically on molybdenum electrodes, the deposit weighing only about 1/10 mgm. X-ray investigations, carried out by the secondary ray method to avoid the possible volatilisation of the substance under the action of the cathode rays, have shown that the deposit cannot contain more than 1/2 per mille of the element looked for. The X-ray line searched for was polonium L_{a_1}, the wave-length of which was calculated from Moseley's law to be 1111 X. U. All the lines on the plate could be identified as belonging to lead, bismuth, silver, mercury, or tungsten. As we started with about 400 grams of each of the minerals mentioned, 1 gm of each mineral cannot contain more than 10^{-11}

gm. of the element in question. This negative result is in agreement with generalisations arrived at by Dr. A. S. Russell.

There is very little hope of finding an inactive polonium isotope, or in general of extending beyond bismuth (83) the series of stable elements.

Comment on paper 15

The search for unknown elements is much facilitated by adding a radioactive isotope of the desired element to the solution of minerals in which the element is most likely to be present. In paper 15 the conclusion was reached that a heavier stable element than bismuth is unlikely to be found. This conclusion is supported by later experience.

In an earlier investigation carried out in 1926 in Copenhagen an unsuccessful search was made for a stable isotope of the element 87 by trying to detect α-rays emitted by $MsTh_2$ or β-rays by radon. Both processes should lead to the formation of the element 87. A radioactive isotope of this element was later discovered by Perey and Lecoin. A more detailed presentation of the results stated in paper 15 is to be found in *Z. f. anorg. Chem.* vol. 194.

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Originally published in *C. R. Acad. Sci., Paris* **178,** 1324 (1924)

16. RADIOCHEMICAL METHOD OF STUDYING THE CIRCULATION OF BISMUTH IN THE BODY

I. A. Christiansen, G. Hevesy and S. Lomholt
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During recent years bismuth has acquired increasing importance in the treatment of syphilis[1]. In order to study the conditions of absorption. distribution in the body and elimination, we have used a radiochemical method which was first proposed by Hevesy and by Paneth[2].

The principle of the method is as follows: The medicament is prepared from a mixture of a bismuth salt solution and a solution containing a radioisotope, radium-E, of this element. It is well known that a final measurement of the quantity of radioisotope present in the sample suffices for calculating the quantity of inactive bismuth.

Radium-E was extracted with a hydrochloric acid solution from radium-D, which had been produced by disintegration of the emanation from a quantity of radium corresponding to 2—4 c. The experiments were performed on rabbits. From time to time small quantities of an oil suspension of the medicament were injected intramuscularly. The rabbits were killed after about 15 days.

The following samples were examined: (1) the places of injection; (2) the most important organs; (3) the daily amount of urine; (4) the daily amount of faeces; (5) small known amounts of the suspension used in the experiment.

All the organic tissues were prepared for analysis by charring with small quantities of fuming nitric acid; the ash was dissolved in dilute nitric acid and the acid was evaporated of in a petri dish; the radioactivity of the small quantity of salts remaining at the bottom of the capsule was finally determined electroscopically by measuring the β-rays of the radium-E. The α-rays from the polonium present in the residue were absorbed by means of an aluminium foil about 0.05 mm thick. Control experiments have shown that the maximum error of the various experiments performed by this method is about 10 per cent.

[1] Sazerac and Levaditi, *C. R. Acad. Sci., Paris* **172,** 1391 (1921); *Ibid.* **173,** 338 (1921).

[2] See Aston, *The Isotopes,* London (1923); F. Paneth, *Z. angew. Chem.* **35,** 549 (1922); G. Hevesy, *Biochem. J.* **17,** 441 (1923).

Nine rabbits were used in these experiments. Quinine bismuth iodide[1] was used in five cases and bismuth hydroxide in the remaining four. The results obtained were in reasonable agreement. We shall limit the results presented here to those from one of the experiments with quinine bismuth iodide. These results are summarized in Fig. 1. The heights of the vertical columns represent the quantities of bismuth injected daily; the shaded parts of the columns represent the quantities found

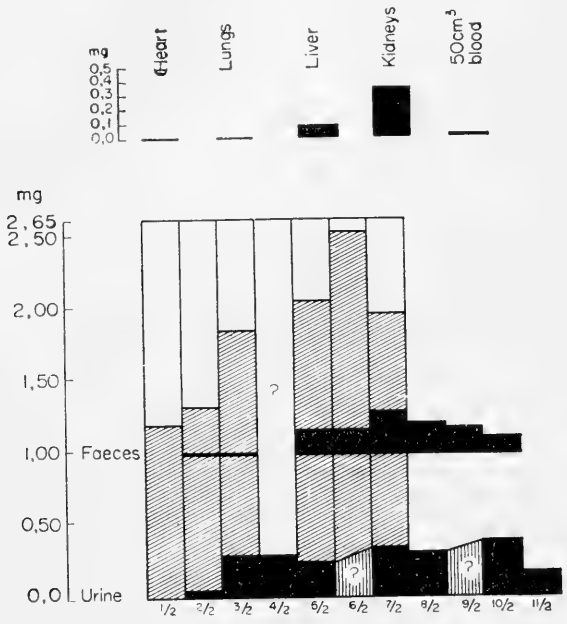

FIG. 1
Distribution of bismuth in the rabbit

at the corresponding points of injection. The upper black columns represent the quantities found in the daily faeces and the lower black columns the quantities found in the urine. The black rectangles placed in the upper part of the diagram give the contents of the various organs.

The general results of all the experiments can be summarized as follows : (1) bismuth is eliminated chiefly in the urine; the quantity of bismuth found in it is double the amount passed in the faecal matter; it increases during the period of treatment in the urine but this is not so clearly demonstrated in the faecal matter; (2) the heart and lungs contain only a small amount of bismuth; the liver contains quite a small quantity and the kidneys a fair amount, generally more than double the amount in the liver; only a very small quantity of bismuth has been found in 50 cm^3 of blood.

The results obtained show that bismuth should only be used with great care, because of its quite slow and irregular distribution leading possibly to a danger of poisoning. Its resemblance with mercury[1] from the aspect of circulation. Precautions are required in the simultaneous use of the two metals, which cause accumulation of the toxic effect.

[1] See Svend Lomholt, *Brit. J. Dermatol.* (1921); *Arch. Dermatolog. u. Syphilis* **126,** 154 (1918).

Originally published in *C. R. Acad. Sci., Paris* **179,** 291 (1924).

17. RADIOCHEMICAL METHOD OF STUDYING THE CIRCULATION OF LEAD IN THE BODY

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At the meeting of the Academy on 7 April 1924 we presented some work performed by a radiochemical method on the circulation of bismuth in the body. Since then we have used the same method for some experiments on the circulation of lead, the first results of which are presented here.

In using bismuth in medicine it is of very great importance to know the rate at which the bismuth injected into the body is distributed and eliminated. This question can be resolved by using a substance with a quite short half-life (5 days), such as radium-E. It is quite different in the case of lead, since all the medical interest in this element centres around the chronic poisoning which is caused gradually by the absorption of small quantities of lead during a long period of time. This is the reason why we have used radium-D (half-life 20 years) in our experiments, whereas Hevesy, in his experiments on the distribution of lead in plants[1], obtained satisfactory results with another isotope of lead, thorium-B, having a half-life of only 11 hr.

Since radium-D emits only soft β-rays, which are difficult to measure with the electroscope, we have counted the β-radiation of radium-E in equilibrium with the radium D and, consequently, have measured the various products of analysis only at the end of several weeks.

The experiments were performed on rabbits and guinea pigs. The method described previously has been modified slightly: Instead of evaporating the solution of the organic matter, which is decomposed by means of nitric acid (or potassium permanganate in acid solution), it is diluted, treated with 100 mgm inactive lead nitrate, and lead sulphide is precipitated. After filtering at the pump on a plane filter, the dried filter is placed in a petri dish and the activity of the deposit is finally determined.

One example only is given here from our experiments. The lead hydroxide, mixed with olive oil and a little carbon black, contained a quantity

1. G. Hevesy, *Biochem. J.* **17,** 441 (1923).

of radium-D derived from the disintegration of an amount of emanation corresponding to ½—1 c. The results are shown in Fig. 1.

The heights of the vertical columns represent the quantities of lead injected daily; the shaded parts of these columns indicate the deposits of corresponding injections. The upper black columns represent the

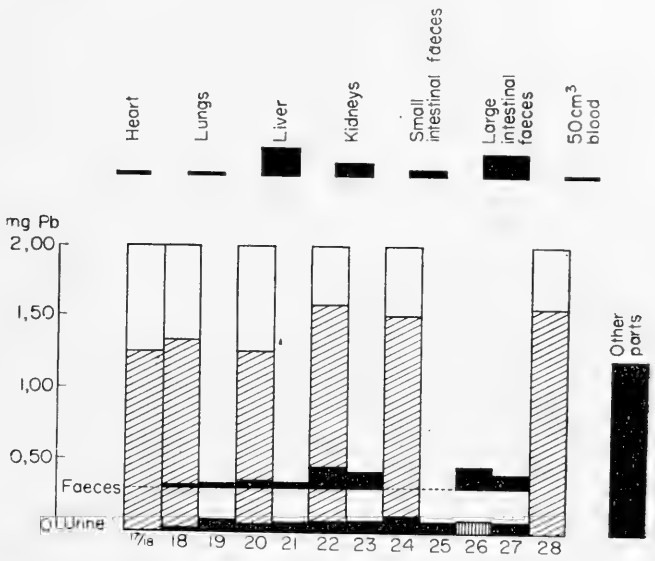

FIG. 1
Distribution of lead in the rabbit

quantities found in the daily faeces and the lower black columns show the quantities present in the urine. The black rectangles in the upper part of the diagram give the contents of the various organs.

It will be evident that there is quite a substantial difference between the results for bismuth and for lead; the amounts of lead stored in the liver and eliminated in the faeces are larger, at the expense of the amounts found in the kidneys and urine which are the major participants in the case of bismuth.

We have recovered 90 per cent of the amount of lead injected.

Comment on papers 16, 17

In the early twenties dermatologists became much interested in the therapeutic application of bismuth compounds. This induced us, together with Christiansen and Lomholt (the latter being a dermatologist) to investigate the distribution of administered bismuth in the rabbit. We applied in our study RaE-labelled bismuth. This work was then extended to the study of the distribution of labelled lead. The first-mentioned investigation was the first application (1924) of radioactive tracers in animal physiology, shortly following their first application in plant physiology.

While participating at the Liverpool meeting of the British Association for Advancement of Science, the writer learned that the gynaecologist Blair Bell obtained good results by applying lead salts in cancer therapy. This induced us (Hevesy and Wagner, 1930) to compare the distribution of lead between normal and cancerous tissue, applying labelled lead. The great Freiburg pathologist Aschoff on my request delegated a Japanese collaborator of his to help us in this work, and later Schoenheimer to assist the latter. This was the first experience of schoenheimer in the field to which he later made, jointly with his eminent colleague Rittenberg, a very great number of unsurpassed classical contributions.

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18. RADIOACTIVE INDICATORS IN THE STUDY OF PHOSPHORUS METABOLISM IN RATS

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Recent progress in the production of radioactive isotopes by neutron bombardment makes the radioactive isotope of phosphorus $^{32}_{15}P$ easily accessible. This isotope, which has a half-life value of 15 days, can be utilised as an indicator of inactive phosphorus in the same way that the radioactive isotopes of lead, bismuth and so on were formerly used

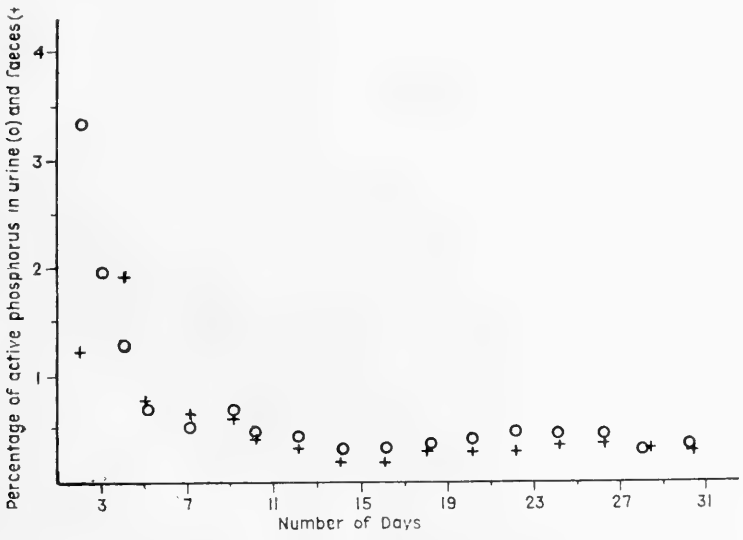

Fig. 1. On the first day 7.4 per cent of phosphorus was found in the faeces and 5 per cent in the urine

as indicators of these elements. If, for example, we add active $^{32}_{15}P$ to 1 mgm of inactive phosphorus in such quantity that the Geiger counter registers 1000 impulses per minute, carry out with the phosphorus activated in this way any sort of chemical or biological reaction and then find that the product obtained gives 1 impulse per minute, we may conclude that 1/1000 mgm of the phosphorus originally introduced is present in the product investigated.

Rats were fed with a few milligrams of sodium phosphate containing $^{32}_{15}P$ as indicator. The radioactive phosphorus present in the urine and faeces was then investigated for a period of a month. The result is shown in Fig. 1, which shows the percentage of the 2 mgm of phosphorus taken, found daily in the excrements. The rat was killed, and, after ignition, the phosphorus content of the different organs was investigated. The result of an experiment in which the rat was killed 22 days after being fed on active phosphorus is seen in the first column in Table 1. The largest part of the phosphorus taken is present in the bones, and the smallest in the kidneys. When, however, we take into account the very different weights of the different organs and calculate the phosphorus content of the latter per gram after drying, we obtain a very different picture, as seen from the second column in Table 1. The spleen, kidneys, and the brain are found to contain per gram most of the active phosphorus. During one of the experiments, the rat produced six offspring on the seventh day, of which five were eaten by the mother; this caused a large increase in the active phosphorus content of the excreta in the following three days. The presence of 2 per cent of the 2 mgm active phosphorus taken by the mother was revealed by the analysis of the remaining offspring.

Table 1. — Distribution of the active phosphorus in the rat

	Per cent	Per cent
Urine	26.3	per gm
Faeces	31.8	
Brain and Medulla	0.5	14.7
Spleen and Kidneys ...	0.2	18.2
Liver	1.7	13.9
Blood	0.4	1.8
Skeleton	24.8	2.8
Muscles and fat	17.4	7.4

The active phosphorus content of the urine and faeces shows great fluctuations during the first few days after the intake of the preparation. Later, it becomes fairly constant; and we have obviously to deal with the excretion of phosphorus which has already been deposited for a while in the skeleton, the muscles, or other organs, and which has been displaced again. From our experiments, it follows that the average time which a phosphorus atom thus spends in the organism of a normally fed rat is about two months. This is also supported by the fact that rats killed about a month after the intake of phosphorus contain only about half the active phosphorus found in those killed after a weeks time. This result strongly supports the view that the formation of the

bones is a dynamic process, the bone continuously taking up phosphorus atoms which are partly or wholly lost again, and are replaced by other phosphorus atoms. In the case of an adult rat, about 30 per cent of the phosphorus atoms deposited in the skeleton were removed in the course of twenty days.

In another set of experiments we investigated the different parts of the skeleton. No conspicuous differences in the active phosphorus content could be found, with the exception of the teeth. The front teeth, which grow rapidly in rats, contained a larger part of the 2 mgm phosphorus taken than the average of the whole skeleton, the ratio being about 10 : 1 in the case of adult and 6 : 1 in that of half-adult rats, whereas the molar teeth took up less than the average per gram of the skeleton, the ratio being 1 : 2 in the most extreme case. A detailed account of these and further results will be published elsewhere.

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19. STUDIES ON THE METABOLISM OF PHOSPHORUS IN ANIMALS

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In a recent letter to *Nature*[1] we communicated the results of some experiments on the metabolism of phosphorus using a radioactive phosphorus isotope as indicator. What follows is a more detailed description of some of our experiments, carried out chiefly on rats but partly also on human subjects.

PRINCIPLE OF THE METHOD USED

Disregarding hydrogen, the only element which is ever met with in a nuclear state (as a proton) in chemical reactions, isotopes do not separate to a measurable extent during chemical or biochemical processes. It follows from this inseparability that when a known amount of radioactive phosphorus is added to, for example, 1 mgm of phosphorus the presence of the former will always indicate the presence of the latter, we can thus distinguish for example between the phosphorus atoms taken in with the food (to which we add some radioactive phosphorus) and those already present in the system. The use of isotopic indicators is not dependent on an absolute inseparability of isotopes by chemical methods. We know indeed that minute separations almost always occur. It is sufficient that, within the analytical accuracy claimed, no separation takes place.

Phosphorus has only one stable isotope ^{31}P but we can prepare unstable radioactive isotopes of phosphorus having atomic weight of 30 and 32; the latter has a half-life of about a fortnight and is very suitable for use as an indicator. It was used by us in many experiments of different kinds.

[1] O. Chievitz and G. Hevesy, *Nature* **136,** 754 (1935).

PREPARATION OF RADIOACTIVE PHOSPHORUS

Radioactive phosphorus $^{32}_{15}P$ can be prepared from chlorine or from sulphur under the action of fast neutrons, or from ordinary phosphorus under the action of slow neutrons; the nuclear reactions are:

$$^{35}_{17}Cl + ^{1}_{0}n = ^{32}_{15}P + ^{4}_{2}He$$

$$^{32}_{16}S + ^{1}_{0}n = ^{32}_{15}P + ^{1}_{1}H$$

$$^{31}_{15}P + ^{1}_{0}n = ^{32}_{15}P$$

Using neutrons liberated from mixtures of radium and beryllium, ^{32}P can be prepared most conveniently from sulphur. We found it advisable to use carbon disulphide instead of the elementary sulphur used by FERMI and his colleagues in their original experiments. About 10 litres of carbon disulphide were exposed to neutrons from radium-beryllium mixtures and a fortnight later the carbon disulphide was distilled off. The residue contained the radioactive phosphorus formed, along with some of the decomposition products of carbon disulphide. The residue was oxidized and the phosphoric acid obtained converted into the phosphate compound wanted. We used chiefly sodium radiophosphate in our experiments. The weight of the radiophosphorus produced is extremely minute; using a source containing 100 mgm of radium, less than 10^{-10} gm of radiophosphorus is obtained. By adding a suitable quantity of sodium phosphate to the sodium radiophosphate solution we obtain the "radioactive" ("labelled") sodium phosphate desired.

To concentrate the radiophosphorus obtained by neutron bombardment of carbon disulphide other methods besides that outlined above were used. A very convenient way to prepare nearly pure radiophosphorus is the following. Under the action of the radiation some decomposition of the carbon disulphide takes place and a partly orange-coloured precipitate is formed which settles on the glass walls. This slight precipitate contains a large part of the radioactive phosphorus formed. The precipitate is possibly identical with the red sulphur described by MAGNUS as far back as 1954, which was found to consist of a mixture of sulphur and organic sulphur compounds. We are engaged on the investigation of this precipitate.

In a third method of preparation the phosphorus formed was removed from the carbon disulphide solution by shaking the latter with diluted (20 : 1) nitric acid.

DETERMINATION OF THE RADIOACTIVE SODIUM PHOSPHATE

The radioactivity of the samples of blood, bones, etc. to be analysed is in most cases too feeble to be measured even by means of a very sensitive electroscope. GEIGER-MÜLLER counters, much more sensitive instruments, are therefore utilised for measuring purposes. We use for the most part tubes having an available surface of about 1.5 cm^2. The sample to be measured must accordingly be spread over about the same area. The β-rays emitted by the radio phosphorus are fairly penetrating and are not much weakened when an aluminium dish of 1.5 cm^2 surface is filled to a depth of a few millimeters with a bone sample weighing 100 mgm. We want to know what percentage of the radioactive phosphorus taken is to be found after a certain time in, for example, the bones. The procedure is as follows. We take a solution of active sodium phosphate, use 99 per cent of it for feeding the animal and keep 1 per cent as a "standard". We kill the animal, separate a bone sample, ignite it, and measure its activity. Should the latter be, for instance, half as large as that of the standard which is measured simultaneously, then we can conclude that 0.99×0.5 per cent of the active phosphorus atoms eaten are actually present in the bone sample investigated. Although the β-radiation from radioactive phosphorus atoms is not much weakened in penetrating through 100 mgm of bone ash, we can entirely eliminate the possible error due to this absorption by adding 100 mgm of calcium phosphate to the standard solution; this has the same absorbing power as the bone sample. It is advisable to make the standard as similar to the sample to be measured as possible. In dealing with urine, faeces, muscles, liver etc. we first destroyed the organic matter by one of the usual methods; in several cases, however, these were replaced by treatment with fuming nitric acid. Then calcium phosphate and calcium oxide were added if necessary to make the sample more similar in its composition to our standard preparation and finally the sample was ignited.

To demonstrate the utility of the isotopic indicator method we will first consider the problem of the origin of the phosphorus in the faeces.

ORIGIN OF THE PHOSPHORUS IN THE FAECES

Chemical analysis enables us to determine the phosphorus content of the excreta but not to decide to what extent the phosphorus found in the faeces is undigested material and what fraction of it is phosphorus having its origin in the organism. The investigations described in this paper have revealed that a fairly rapid interchange takes place between

the phosphorus present in the different bodily organs and that present in the blood. A part of the latter finds its way, when the digestive fluids are formed, into the intestinal tract and is thus added to the faeces. The following experiment permits us to distinguish between food phosphorus and that originating from the blood. We add a known amount of radioactive phosphorus to the diet and determine what percentage of the latter is to be found in the faeces. In a separate experiment we inject a known amount of radioactive phosphorus (sodium phosphate) into the blood and determine what part of this phosphorus appears in the faeces. The combination of the two results enables us to determine what part of the phosphorus found in the faeces is due to incomplete digestion of the food eaten.

In Table 1 the amount of radioactive phosphorus eliminated through the kidneys and the gut is given for the case of a patient fed on a normal hospital diet to which 0.5 mgm of labelled sodium phosphate was added. Within 5 days 21.7 per cent of the phosphorus was eliminated

TABLE 1. — RADIOACTIVE PHOSPHORUS GIVEN TO HUMAN SUBJECT PER OS

Number of days after taking P	Diuresis in gm	Percentage of original radioactive P	
		in 1 gm of the urine ash	in total urine
0—1	1880	1.23	11
1—2	1800	0.31	2.8
2—3	1620	0.31	2.8
3—4	1670	0.26	2.4
4—5	1540	0.29	2.7
5—6	1860	0.25	1.8
			in total faeces
0—1	—	—	0
1—2	—	—	7.0
2—3	—	—	5.6
3—4	—	—	1.8
4—5	—	—	1.1
5—6	—	—	0

in the urine and 15.5 per cent in the faeces. Similar results were obtained in other cases. Table 2 shows the results obtained when the radioactive phosphorus was injected into the blood of the same patient. Within days 20.5 per cent was lost through the kidneys and 2.5 per cent through the gut. Thus about 1/8 of the phosphorus atoms eliminated from the blood pass through the gut. By combining the above results it follows

that of the phosphorus found in the faeces about 20 per cent was not undigested material but was phosphorus which had already had a share in building up the organism and had left it by entering the digestive liquids and thus getting into the faeces.

In the case above, 22.3 per cent of the radioactive P left through the kidneys within 6 days and in other cases values varying between 20 and 25 per cent were obtained.

TABLE 2. — RADIOACTIVE PHOSPHORUS INJECTED INTO THE BLOOD OF A PATIENT

Number of days after injection	Diuresis in gm	Percentage of original radioactive P	
		in 1 gm of the ash	in total urine
0—1	1650	0.78	12.5
1—2	1510	0.20	3.1
2—3 / 3—4	1850	01.5	2.9
4—5	850	0.16	1.6
5—6	1450	0.13	2.2
7—8	800	0.09	0.6
8—9	2000	0.10	1.8
		in 1 gm of the ash	in total faeces
0—1	—	0.085	0.24
1—2	—	0.11	1.37
2—3	—	0.072	0.37
3—4	—	0.072	0.56
4—5	—	0	0

In carrying out experiments like those described above, the most satisfactory procedure would be to replace by radioactive labelled phosphorus atoms the normal phosphorus present in all the foodstuffs administered. By bombarding the material in question with a strong source of slow neutrons we could turn some of the phosphorus atoms into radioactive phosphorus; but such a process always leads to a disruption of the molecular bonds of the phosphorus atoms which become activated and so to a destruction of the chemical compound. We must therefore content ourselves with adding inorganic radioactive phosphate to the food consumed and try to obtain a mixture of radioactive inorganic phosphate and food as uniform as possible. In our experiments carried out with human subjects the sodium radiophosphate was administered in a large volume of milk. Milk contains 0.0795 per cent of inorganic phosphorus and about half that amount (0.036 per cent)

of phosphorus in organic form. Although the latter does not exchange with the atoms of the inorganic radioactive phosphate, the bulk of the phosphorus (0.0795 per cent) reaches a state of kinetic equilibrium with the radioactive phosphate added and becomes radioactively indicated. During the digestion process the 0.036 per cent will be set free from its molecular binding and only at this stage will it have an opportunity to become thoroughly mixed (in an atomic sense) with the radioactive phosphate atoms. While, as has already been mentioned, it would be preferable in investigating phosphorus metabolism to utilize food in which all the phosphorus atoms are labelled, it is not probable that the information obtained with such material would be appreciably different from that obtained in the experiments described in this paper. Experience shows that the retention of phosphorus does not depend on the form in which the phosphorus is present[1] in the food, on whether it is present as inorganic and thus exchangeable phosphate or as non-exchangeable. Ducks reared on diets containing phosphate only in inorganic form matured normally and laid 85 to 795 eggs during the first summer[2]. About 15 per cent of the phosphorus present in meat, more than half that present in milk, and the greater part of that present in vegetables, i.e. the bulk of the phosphorus eaten, is present in inorganic and thus exchangeable form.

Rats are inclined to eat their offspring and they could easily be fed on young rats born by a mother fed on radioactive phosphorus, but the chief source of phosphorus would in this case, too, be inorganic phosphorus, namely that present in the skeleton.

ELIMINATION OF PHOSPHORUS BY RATS

We carried out numerous experiments with rats which were fed on a normal diet to which radioactive phosphorus was added. In some cases we added 0.1 mgm or less in the form of sodium phosphate dissolved in a few drops of water which was then soaked up by a small piece of bread given to the animal. The average of several experiments gave a total excretion of 26 per cent through the kidneys and of 32 per cent through the gut. In some other experiments calcium phosphate was administered, mixed with butter, which was given to the rat on a small piece of white bread. The result of such an experiment is seen in Table 3, which contains the results of the analysis of the urine and the faeces collected during 19 days. The urine was concentrated by evaporation, treated with fuming nitric acid, and ignited; a known fraction of the ash

[1] M. Speirs and H. C. Sherman, *J. Nutrit.* **11,** 216 (1936).
[2] G. Fingerling, *Biochem. Z.* **38,** 448 (1911).

obtained was then introduced under the Geiger counter. 19 days later the rat, which weighed 256 gm, was killed, the corpse was treated with fuming nitric acid to destroy organic compounds, the fatty residue was treated with conc. sulphuric acid, and then ignited in an electric

TABLE 3. — 1.5 MGM RADIOACTIVE CALCIUM PHOSPHATE ADDED TO NORMAL DIET OF ADULT RAT

Number of days after taking rad. P	Percentage of original rad. P	
	in the urine	in the faeces
0— 3	11.4	13.1
3— 7	3.9	4.7
7—10	2.7	2.4
10—13	1.8	0.93
13—16	1.3	1.1
16—19	1.2	1.88
Total	22.3	24.0

[1] Faeces contaminated by urine.

oven. 50.2 per cent of the phosphorus given was found in the ashes, which were to a large extent composed of calcium phosphate, and had a total weight of 5.84 gm.

In some cases we added large amounts of calcium phosphate containing active phosphorus to the diet. When for example 18 mgm of phosphorus as calcium phosphate were given — this corresponds to about four times the phosphorus present in the normal diet — 41 per cent of the active phosphorus was eliminated through the gut in the course of 19 days and only about 10 per cent through the kidneys. Furthermore an analysis of the active phosphorus content of the corpse and the excreta revealed that when large amounts of phosphorus were added to the diet the animals would eat only part of it, however, carefully it was administered. We decided therefore to study the effect of the intake of large amounts of phosphorus on dogs.

The phosphorus atoms absorbed have ample opportunity to enter into kinetic exchange with the phosphate ions present in muscles, bones, and other organs and also to a certain extent to enter organic molecules and replace the phosphorus atoms present there. Many of the last mentioned processes are dependent on enzymatic action. The rate at which the active phosphorus enters the blood corpuscles, the particulars of this process, and the distribution of the radioactive phosphorus between the blood and the different organs were investigated by Professor LUNDSGAARD and one of us and the results will be published shortly.

PHOSPHORUS EXCHANGE IN ADULT RATS

A preliminary investigation revealed the following distribution in adult rats killed three weeks after eating the radioactive phosphate administered in the form of 0.5 mgm sodium phosphate added to the normal diet.

TABLE 4. — DISTRIBUTION OF RAD. P IN ADULT RATS KILLED 3 WEEKS AFTER EATING IT

	p. c. rad. P
Urine	26.3
Faeces	31.8
Skeleton	24.8
Muscles and fat	17.4
Liver	1.7
Brain and Medulla	0.1
Kidneys and Pancreas	0.1

In interpreting the results obtained it is convenient to compare the radioactivity of equal weights (say 100 mgm) of the ashes, of the bones, the teeth, the liver, and so on. These all contain about the same percentage of phosphorus (17 per cent, 17 per cent, 16 per cent); the phosphorus content of the ash of the blood is rather different, but as was stated above the behaviour of the active phosphorus in the blood was not investigated to any great extent in the course of this work.

In a series of experiments we gave the same amount of radioactive phosphorus to 6 rats. One pair of rats was killed after one week, a second pair after two weeks, and a third pair after three weeks. The results are seen in the following table.

The weights of the different skeletons vary to an appreciable extent; the weights of the animals were 225, 210, 200, 215, 235 and 220 gm before, and 220, 205, 200, 205, 235 and 220 gm resp. after the experi-

TABLE 5

Animal killed weeks after eating rad. P	p. c. of rad. P found	
	in the skeleton	in the incisors
1	34.2	2.1
1	35.3	2.1
2	32.2	2.8
2	27.2	2.1
3	24.6	2.8
3	25.4	2.7

ment. In comparing the rad. P content of different organs of the same rat we are independent of the assumption that all the rad. P given was actually eaten by the animal, though we are not, when we compare the rad. P content of organs from different rats. The greater rad. P content of the bones of the animals killed after the lapse of only a week cannot, however, be due chiefly to such a reason as this, because in that case the rad. P content of the incisors would also be appreciably higher in the case of rats killed after the lapse of one week. This is not the case, as can be seen from the figures in Table 5. We must therefore conclude that the rad. P taken up by the bones, and in exactly the same way all the phosphorus taken up by the bones, has a certain chance of being lost again. Indeed an uptake of phosphorus atoms by the bones of an

Table 6

	p. c. of rad. P taken, present in 100 mgm of ashes	weight of ashes of the organ in mgm	p. c. of rad. P taken, present in the total ashes
a) rat killed after 1 Week			
Bones	0.8	4300	34.3
Molars	0.2	100	0.2
Incisors	1.3	253	3.3
Liver	3.2	103[1]	—
b) rat killed after 2 Weeks			
Bones	0.7	4200	29.5
Molars	0.2	100	0.2
Incisors	1.9	215	4.1
Liver	2.0	210	4.2

adult rat can only be explained by a corresponding process in the opposite direction. Another example of the decrease in the active phosphorus content of the bones with time is seen in Table 6.

While the bones show a decrease in their rad. P content with time and the molars no change to within the accuracy of experiments, the incisors show a marked increase. The incisors of adult rats show a very pronounced growth. The discussion of their behaviour is therefore better postponed and will be dealt with in the next chapter, where experiments on young rats are described.

The results of an experiment carried out with two rats both killed after 5 days time are seen in Table 7.

[1] The weight of the ashes of the liver was found to be very variable.

TABLE 7

	p. c. rad. P taken found in 100 mgm of ashes	
	I	II
Bones	1.3	1.4
Molars	0.24	0.34
Incisors	2.4	2.3
Liver	2.7	1.7
Muscles	1.7	1.8
Brain	0.46	0.58

As is seen from the above figures the muscles show a somewhat larger content of rad. P than an equal weight of the bones. The active P content of the brain ash is decidedly lower. To ascertain if the phosphorus atoms present are not acid soluble, phophorus compounds are also replaced by active P atoms, and the brain treated with 6 per cent trichloracetic acid solution. By this means all the acid soluble phosphorus was removed. The operation was carried out with great care. After igniting the filtrate and residue, the activity of both fractions was measured. We found both fractions to be active, the activity of the phosphatide fraction being about $^1/_3$ of that of the trichloracetic acid extract. We are engaged in following up this point in greater detail, using more trustworthy methods of separation.

EXCHANGE OF PHOSPHORUS BY GROWING RATS

The uptake of phosphorus shown by different organs of rats about 2 weeks old is seen in Table 8. The rats were killed three days after being fed with radioactive phosphorus added to their normal diet.

Focusing our attention first on the bones we notice that 100 mgm of ash contain more than ten times as much radioactive phosphorus as

TABLE 8

	Rat I (weight 27 gm)		Rat II (weight 24 gm)	
	weight of ashes in mgm	p. c. of rad. P taken present in 100 mgm of ashes	weight of ashes in mgm	p. c. of rad. P taken present in 100 mgm of ashes
Bones (Leg)	65.4	10.5	59	10.9
Incisors	—	5.8	—	5.8
Molars	39.4	2.9	33.8	2.8
Muscles	—	11.0	—	—
Blood	—	2.8	—	2.6

was found in the case of adult rats. The high radioactivities of the bones are due to the fact that in this case an appreciable part of the bones are actually grown from blood of high radioactive phosphorus content; a rapid formation of new cells takes place, in whose building up radioactive phosphorus participates.

A very conspicuous difference is found between the active phosphorus content of the molars of rapidly growing and of adult rats, the great difference being due primarily to the low exchange values in the latter.

The brain as a whole was found to contain 0.5 per cent of the active phosphorus taken by the animal.

The ratio between the rad. P content of the muscles and the bones is nearly unity in the case of the young rats, while in adult rats the muscles show a higher rad. P content.

When we compare the radioactive phosphorus content of the bones of growing rats, we find for example more activity in 100 mgm of the ashes of the bones of animals killed after one week than in those killed after two weeks. This is due chiefly to the fact that the phosphorus atoms present in the bone at a certain time will soon be found in an entirely new part of the growing skeleton, and will also have a certain chance of leaving the skeleton entirely. If we want to obtain information on the latter point we must compare the "radioactive" phosphorus contents of whole skeletons. We carried out such experiments, comparing the whole of the leg material. Five very young rats having a total weight of only 25 gm were fed on their normal diet plus some radioactive phosphorus (0.50 mgm each). Two were killed 2 days later and three 65 days later. 10 mgm of the ashes of the leg bones of animals killed after 2 days contained 8.4 times as much radioactive phosphorus as that of rats killed after 65 days. The active phosphorus atoms were in fact distributed all through the greatly increased amount of bone tissue; the leg bones increased in the course of 63 days to about ten times their original weight, as can be seen from Table 9. When we compare the radioactive phosphorus content of the total bone material of the legs, the difference between the rats killed after 2 days and after 65 days is much less; the difference still present is due to the loss of phosphorus atoms by the bone material. The phosphorus atoms which were present in the bone for a while and left it again will be found partly in the excrements but to some extent also in some of the organic compounds building up the organism. In the course of two months about one third of the phosphorus atoms originally present left the skeleton.

A comparison of the behaviour of the active phosphorus present in the incisors with that in the bones is difficult in view of the rapid using up and replacement of the incisors. Prof. HOLST, Prof. KROGH

and one of the writers of this paper are at present engaged on an investigation of the exchange of phosphorus in the incisors on different lines.

Table 9

Period between taking of radioactive P and killing	Weight of bone ash (legs) in mgm	p. c. of radioactive P present
2 days	65.4	7.4
2 „	59.0	7.5
65 „	440	4.1
65 „	514	5.1
65 „	613	5.5

UPTAKE OF PHOSPHORUS IN PREGNANT RATS AND IN HUMAN PLACENTA

In Table 10 the result of the investigation of adult normal and pregnant rats is seen. Those designated I were killed after a lapse of one week, those marked II after two weeks.

As can be seen from the above figures the different organs of the pregnant rats took up less rad. P than normal rats, the difference being found at least partly in the foetus and placenta. In the first rat, which was in an advanced stage of pregnancy, the foetus and still more the placenta had a high content of rad. P, higher than any organ of the mother, We find here again a very conspicuous illustration of the difference between the taking up of P through an exchange process and through

Table 10

	Normal rat p. c. of rad. P taken present in 100 mgm ashes	Pregnant rat p. c. of rad. P taken present in 100 mgm ashes
I Bones	0.78	0.49
II Bones	0.74	0.52
I Incisors	1.3	1.2
II Incisors	1.9	1.7
I Molars	0.21	0.12
II Molars	0.23	0.16
I Liver	2.0	1.6
II Liver	1.94	1.0
I Foeta	—	2.7
II Foeta	—	0.54
I Placenta	—	4.0
II Placenta	—	2.3

actual growth, the latter being much more effective in introducing rad. P into the tissue. An appreciable part of the foetus has actually been built up by utilising the circulating rad. P and has correspondingly a high ^{32}P content. This is still more the case for the rapidly growing placenta. In the case of the second animal, pregnancy occurred at a much later date than the intake of rad. P. The foetus was nourished by blood poor in rad. P, and correspondingly the rad. P content of the ash of the foeta was much less. Whereas in the first case the weight of all foeta was 345 mgm, in the second case it was only 52 mgm, the weight of the placenta ash being 43 and 12 mgm respectively.

We also had an opportunity to find what was a comparatively very high rad. P content for the placenta of a human subject; as much as 0.095 per cent was found in the ash of the placenta, which weighed 133.8 mgm. We can estimate the total ash which the patient in question should give on ignition as 2800 gm. The weight of the placenta ash thus amounted to less than $^1/_{20000}$ of the total ash, while the rad. P content was as much as $^1/_{1000}$ of the total amount of rad. P given, showing a concentration of rad. P in the placenta ash more than twenty times as great as that in the average ash of the body. One might try to explain the high rad. P content of the placenta by its high blood content. That this explanation fails is seen, however, from the following. The ash of the placenta was found to weigh 133.8 mgm and the ash of about 5 cc. of blood would weigh the same. But as early as 8 hours after the injection of rad. P such a volume of blood was found to contain less than $^1/_{10000}$ of the latter[1], and after the lapse of a few days — when the placenta were removed — still less. The high rad. P content of the placenta cannot therefore be due to their blood content. No activity could be detected in the ash of the few weeks' old foetus removed in the course of an operation, but the weight of this sample amounted to only a few mgm.

UPTAKE OF PHOSPHORUS BY RACHITIC RATS

We carried out a set of experiments on two months' old rachitic rats, which had been used by Frederica and Gudjonson in their experiments on the effect of vitamin A and D deficiency on rickets. The rats were fed before and during the experiments on a diet free from or poor in vitamins A and D. The weights of the animals before the experiment were 89, 83, 85, 93, 90, 95 and 103 gm. The results are seen in Table 11

[1] In the case of another subject we found 1 cc. of blood to contain 0.0027 per cent of the phosphorus injected after the lapse of 12 hours, the blood corpuscles containing 11 times as much active phosphorus as the plasma.

TABLE 11

Killed	p. c. from the rad. P taken found in 100 mgm ashes				Weight in mgm			
	Bones	Incisors	Molars	Liver	Bones[1] (legs)	Incisors	Molars	Liver
1 Week	4.2	3.8	0.7	3.2	358	100	72	135
1 „	4.2	3.8	1.1	5.9	329	105	76	103
2 Weeks	3.0	4.1	0.9	5.0	403	113	55	86
2 „	3.5	3.7	0.9	5.0	361	96	64	84
3 „	2.7	5.0	1.4	1.8	313	115	69	168
3 „	2.2	3.6	1.1	1.2	419	109	57	145
3 „	2.9	4.3	0.9	1.8	422	115	81	205

The above bone figures show a marked difference as compared with normal rats of the same age (cf. Table 6). We are engaged in carrying out further experiments on rats with rickets.

GENERAL CONSIDERATIONS

The rapid entrance of the labelled phosphorus into the bone is in no way puzzling. If solid calcium phosphate, one of the chief constituents of the bone, is in contact with the solution containing labelled phosphate ions a rapid distribution of the latter takes place between the surface of the solid phase and the liquid phase, as was seen from the following experiment. 3950 gm freshly precipitated $Ca_3(PO_4)_2$ were shaken with 5 cc. of water saturated with $Ca_3(PO_4)_2$ at room temperature and containing an infinitely small amount of labelled sodium phosphate. After lapse of four hours 84.1 per cent of the labelled phosphate ions were found in the solid phase and only 15.9 per cent in the solution. The calcium phosphate of the bone tissue being in a very intimate contact with the blood stream, i.e. with cells containing labelled phosphate, a similar exchange to that described above will take place between the unlabelled phosphate of the bone and the labelled phosphate present in the liquid phase.

Beside the above mechanism we have to consider two others just as important. During growth, the bone tissue formed will be built up from labelled phosphorus as long as the blood stream contains the latter.

Finally we have to envisage a third possibility, namely the entrance of labelled phosphorus into the bone through a constant break-down

[1] The weight of the total skeleton is obtained by dividing the figures obtained for the legs by 0.26.

of the bone tissue already formed and the formation of new tissue in the case of adult animals as well.

The following examples may help to make the three ways of entrance of the labelled atoms into the bone easier to understand.

1) When solid salts are in contact with labelled ions of the solution within a short time a distribution equilibrium of the labelled ions between the surface layer of the solid and the solution will take place, as is seen for example in the experiment described above. This phenomenon was studied extensively by PANETH and his collaborators[1] in the case of lead salt which were shaken with solutions containing labelled (radioactive) lead ions.

2) If we deposit for example lead electrolytically from a solution containing labelled lead ions, the metallic deposit will be a labelled one, just as the bone grown from blood containing labelled phosphorus will contain labelled phosphorus.

3) In investigating the exchange between metallic lead and a solution of labelled lead ions, or vice versa, we find[2] a different behaviour to that described above in the case of lead salts. The exchange in the case of metal is not restricted to the uppermost atomic layer of the lead surface; many atomic layers are involved in the exchange process. This is due to the fact that the lead actually goes into solution from certain parts of the surface, while lead ions are discharged, at other parts. This is a much more effective process in bringing about an exchange between the lead atoms in the solid and in the liquid phase than that observed in the case of solid salts where only the uppermost atomic layer is involved (within any resonable time) in the exchange process. The entrance of labelled phosphorus into the bone will also be much facilitated if it is not only the uppermost phosphate layer that is involved in the exchange process; if in fact the bone is destroyed at certain places and rebuilt at others. In view of the important enzymatic actions[3] going on in the bone tissue such a reversible breakdown process will easily occur.

Summary

By adding radioactive phosphorus (phosphate) to the diet of rats, the metabolism of the phosphorus atoms taken in with the diet can be followed up in the animal body. An appreciable part of the phosphorus taken finds its way not only in growing but also in adult animals into the bones, teeth, muscles, and different bodily organs.

[1] F. PANETH and W. VORWERK, *Z. phys. Chem.* **101,** 445, 480 (1922).

[2] G. HEVESY, *Phys. Z.* **16,** 52 (1915)

[3] R. ROBISON, *The Significance of Phosphoric Esters in Metabolism.* New York (1932).

In growing animals it was found that the atoms already present at an early stage of the formation of the skeleton become distributed in the course of time over the different parts of the skeleton and other organs demonstrating thus the dynamical nature of the building up of bone tissue. Some of the phosphorus atoms present in the bones leave the skeleton for good, being eliminated through the kidneys or the bowls or becoming located in other organs of the body.

The replacement of individual phosphorus atoms by other phosphorus atoms also takes place in the bone tissue of adult animals including that of the teeth.

It was ascertained that about one-seventh of the phosphorus found in the faeces of a human subject is due to material which has entered the intestines through the digestive juices after being located in the blood stream or in the organs of the body for a shorter or longer time.

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20. INVESTIGATIONS ON THE EXCHANGE OF PHOSPHORUS IN TEETH USING RADIOACTIVE PHOSPHORUS AS INDICATOR

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ANATOMICAL INTRODUCTION

The hard part of a tooth is composed of three distinct substances viz. the dental substance proper, dentine, the enamel, and the cement. The dentine constitutes by far the largest portion; the enamel is found in a comparatively thin layer partly covering the dentine; and the cement covers the surface of the root in a thin layer. In the case of the canines of cats we found the weight of the enamel ash to be 11.2% of that of the dentine ash, the weight of the enamel before ashing being equivalent to about 9.7% of that of the dentine.

The dentine is penetrated throughout by fine tubes (dentinal tubes) starting from that side of the dentine which faces the pulpa cavity; they have an initial diameter of 2 to 8μ and do not much diminish in size at first as they approach the surface; the distance between adjacent tubules is about two or three times their width. From the tubules numerous immeasurably fine branches are given off and penetrate the hard intertubular substance. Near the periphery of the dentine, the tubules, which by division and subdivision have become very fine, terminate imperceptibly in free ends. It is reported that tubules have been observed passing into the enamel in the teeth of marsupial animals, and to a less marked degree in human teeth. In this case they pass, not into the enamel prisms, but into the inter-prismatic substance. The enamel is made up of microscopic columns, very hard and dense, arranged close side by side, and fixed at one extremity on to the subjacent surface of the dentine. The enamel columns have the form of six-sided prisms. Their diameter is about 0.005 mm. They are united by a small amount of substance which appears to be similar to the intercellular substance of an epithelium. The small amount (about 1%)[1] of

[1] I. H. Bowes and M. M. Muray, (1935) *Biochem. J.* **29,** 12, 2721.

organic matter in the enamel is probably found to a large extent in the above mentioned connective substance. In marsupials and some rodents there are regular canaliculi in the interprismatic substance.

The central cavity of a tooth is occupied by a soft and very vascular dental pulp, containing cells, blood-vessels, nerves, and fine connective tissue fibres. The cells are partly disseminated in the matrix and partly form a stratum at the surface of the pulp. These superficial cells, the odontoblasts, send out elongations into the tubules in the dentine. It is through the intermediary of the pulp that constituents of the blood get into the hard tissues of the teeth.

Chemical composition of the teeth

a) Dentine.

On analysing a great number of dry human dentine samples Bowes and Murray[1] found a loss in weight of the fresh tissue on ignition amounting to 29—29.7%. The losses on ignition found in some of our experiments can be seen in Table 1, in which we have also included for the sake of comparison the values found for the tibia and jaw.

Table 1. Albino Rat 200 g

Organ		Loss on ignition in % of fresh weight
Incisors	Proximal end	33.6
	Distal end	25.0
	Average	26.4
Molars		27.0
Tibia	Head	79.1
	Average	63.2
Cat 4 kgm		
Incisors		32.0
Canine		35.0
Molar		38.0
Jaw		50.4
Tibia epiphysis		66.8
Tibia diaphysis		36.7

The average values found for the chief constituents of the dentine by Bowes and Murray[1] are seen in Table 2.

[1] I. H. Bowes and M. M. Muray, (1936) *Biochem. J.* **30**, 1977.

Table 2. Analyses of Dentine of Human Teeth
(% in Dry Dentine)

	Slight hypoplasia	Severe hypoplasia
Ash	71.09	70.28
Ca	27.79	26.96
P	13.81	13.5
CO_2	3.18	3.10
Mg	0.835	0.728
Cl	—	0.023

Bowes and Murray give the following average figures for the composition of the enamel:

Table 3. Analyses of Enamel of Human Teeth

	Slight hypoplasia	Severe hypoplasia
Ash	95.38	94.67
Ca	37.07	35.81
P	17.22	17.72

	Slight hypoplasia	Severe hypoplasia
CO_2	1.952	2.434
Mg	0.464	0.477
Cl	0.3	0.19
Fe	0.25	—

As is seen from the above figures phosphorus is the second most abundant mineral constituent of the teeth, its share in the dentine amounting to 13.5—13.8% and in the enamel to 17.2—17.7% while in the dentine ash 18.2—18.4% in that of the enamel 18.4—19.4% were found. In the ash of the incisors of rats an even higher phosphorus content of 20% was found. Bone ash contains an only slightly lower amount of phosphorus than tooth ash, the values found varying between 17.9 and 18.5%.

In distinction to the chief constituents of the teeth the minor constituents vary within wide limits. The composition of the mineral constituents of the teeth corresponds approximately to a mixed crystal of the minerals hydroxide-apatite and carbonate apatite, the former predominating strongly. As in apatite minerals the OH^- ions of the tooth apatite can be replaced to a certain extent by F^- ions for example. The degree of replacement of OH^- by F^-, will depend primarily on the fluorine content of the blood during the development period of the tooth and also on that which circulates in the fully calcified tooth. The fluorine

content of the blood will depend on the fluorine content of the food and water taken up. It is thus easy to explain why the fluorine content of the teeth varies within wide limits (see Table 4). The high fluorine content of the teeth of human beings living at Colorado Springs is due to the high fluorine content of the water which amounts to up to 2 mgm per liter. The high fluorine content of the teeth of some North African sheep is to be explained by the high fluorine content, above 0.02%, of the soil on which they graze. On such soil plants of high fluorine content grow, are eaten by the sheep, and lead to an abnormally high fluorine of the blood plasma, which in turn leads to an abnormally high replacement of OH^- by F^- in the teeth[1, 2, 3, 4].

Table 4. Fluorine Content of Teeth Ash

		%
Man[1]	teeth	0.03
Marine animals	teeth	0.69—0.74
Rats[2]	teeth	0.006—0.03
Man[3] New York	dentine	0.065
Man New York	enamel	0
Man[3] Colorado Springs	dentine	0.112
Man Colorado Springs	enamel	0.065
Man[4]	dentine	0.030
Man[4]	enamel	0.005
Calves[4]	dentine	0.022
Calves[4]	enamel	0.0057
Sharks[4]	teeth	0.89
Sheep, young from neighbourhood of Norwegian aluminium factory where fluorides are utilised[4]	incisors	0.45—0.49
Sheep[5] North Africa	teeth	0.04
Sheep[5] North Africa, attacked by fluorine disease	teeth	0.32—0.45

The much higher fluorine content of animals living in sea water is also due to the comparatively high fluorine content of the latter. In the same way that F^- replaces OH^- in the apatite lattice, magnesium for example replaces calcium. Human dentine ash has a magnesium content[6] of 1.18—1.39% whereas human enamel ash has only 0.42%. While the calcification of the tooth tissue is presumably the result of specific cell activity, it is quite possible that later on a replacement of

(1) R. Klement, *Naturwiss.* **21,** 662 (1933).
(2) R. R. Sharpless and E. V. McCollum, *J. Nutrit.* **6,** 163 (1933).
(3) H. Boissevain and W. F. Drea, *J. Dent. Res.* **13,** 495 (1933).
(4) K. Roholm, *Fluorine Intoxication*, p. 260. Copenhagen (1937).
(5) M. Gand, A. Chavnot and M. Langlais, (1934.) *Bull. Inst. Hyg. Maroc.* Nos. I—II.
(6) M. M. Murray, *Biochem. J.* **30,** 1568. (1936.)

calcium by, for example, magnesium takes place, governed chiefly by solubility (chemical affinity) conditions, and it is quite conceivable that in the course of time more and more calcium is replaced by magnesium if the magnesium: calcium ratio is in favour of such an exchange. The enamel being characterised by a decidedly poorer lymph circulation than the dentine, the much lower magnesium content of the former can easily be accounted for by a reference to the above considerations. The 0.42% magnesium found in the human enamel possibly got into the latter wholly or to a large extent during the formation of the enamel tissue. The presence of as much as 4% of magnesium in elephant dentine is possibly due to a high magnesium content of its food or to a high magnesium retention in its blood. It is also of interest to remark that the magnesium content of the teeth found in prehistoric skeletons is only one third of that found in teeth of recent generations, furthermore that carious teeth[1] show a greatly increased magnesium content. Besides the elements discussed above, spectroscopic investigation[2] revealed the presence of traces of Na, Ag, Sr, Ba, Cr, Sn, Zn, Mn, Ti, Ni, V, Al, Si, B and Cu in dental tissue.

That the concentration of the minor constituents of the teeth does not fluctuate between still wider limits is due to the narrow limits within which the concentration of most elements in the blood plasma is restricted. This is caused partly by a prevention of the resorption of excessive quantities of the elements, conspicuously shown in the case of calcium, and partly by prompt removal chiefly through the kidneys of excessive amounts of the mineral constituents present in the plasma. But even in spite of this levelling mechanism of the blood plasma some of the mineral constituents are deposited to a remarkable extent in the tooth tissue, as is seen above in the case of fluorine, and it is quite possible that even an excessive replacement of, for example, the calcium by magnesium, sodium, or potassium might lower the resistance of teeth to disease.

While the conclusions given above are based partly on hypothetical assumptions, in the case of lead, which also replaces calcium in the crystal lattice, the accumulation in the teeth with time can clearly be shown. While small children have only negligible amounts of lead in their teeth, the lead content increases with age[3], the increase being markedly greater in the case of carnivorous than herbivorous animals, presumably on account of a greater lead intake in their normal nourishment. In the case of lead poisoning the lead content of teeth is greatly

(1) T. Francia, *Ann. Clin. Odoniat.* **8**, 695, (1931); M. M. Murray and J. H. Bowes, *Brit. Dent. J.* **61**, 473, (1936).
(2) E. Lowater and M. M. Murray, *Biochem. J.* **31**, 837, (1937).
(3) F. Perieme, (1934) *Arch. f. Hyg.* **111**, 232.

increased. All these observations support the hypothesis, that even in fully formed teeth an exchange of mineral constituents is regularly taking place. To test this hypothesis we have studied the exchange of phosphorus by means of labelled phosphorus atoms.

PHOSPHORUS EXCHANGE IN TEETH

We investigated the movement of the phosphorus atoms both in the teeth of fully grown and growing animals by using labelled phosphorus atoms as an indicator. By adding radioactive phosphorus, prepared from sulphur by the action of neutrons, to food administered to animals at a known date, it is possible to distinguish the phosphorus atoms which were present in the food sample and which have been retained and deposited in the organism, from those already present in the body and the teeth at the start of the experiment. We can thus follow the movement of the phosphorus atoms taken in for example a glass of milk and investigate if and to what extent these particular atoms get into the teeth and how they are distributed there.

The dentine contains 14% and the enamel 17.5% of phosphorus in the form of phosphate (PO_4). It is the movement of these phosphate radicles which we actually investigate. For the sake of brevity we shall often use the word phosphorus in discussing the behaviour of the phosphate radicle. We may recall that the phosphorus taken with food, amounting in the case of an adult to somewhat more than 1 gm. per day, is to a large extent (in most cases up to about 80%) absorbed from the gut and gets into the blood stream. Adult human blood contains 44—50 mgm% of phosphorus of which only 2—5 mgm% are present as inorganic P. Very different views have been put forward on the formation of the bone and tooth tissue, but they all consider the blood plasma as saturated or nearly saturated with calcium phosphate and the precipitation of the latter from the plasma as being of paramount importance for the ossification process. The solubility of calcium phosphate in the plasma is very strongly affected by the presence of proteins, carbonate and bicarbonate ions, and possibly also other constituents. It is also dependent on the acidity of the blood, slight changes in which may be sufficient to produce precipitation. It seems very probable that it is not simple calcium phosphate but a complex salt of the apatite type, a solid solution of hydroxide apatite and carbonate apatite, that precipitates.

In addition to the inorganic phosphate, blood contains a phosphoric ester at a comparatively high concentration which is mainly found in the corpuscles; as it cannot yield phosphate ions by dissociation, this ester does not affect the saturation of the blood with respect to calcium

phosphate. However, as ROBISON[1] discovered, the cartilage and osteid contain an enzyme, phosphatase, which hydrolyses this ester, thus setting free inorganic phosphate, whereby the concentration of the phosphate ions increases and a supersaturation occurs, followed by a precipitation of the calcium phosphate in the matric of the tissue. With the discovery of the bone phosphatase a second agency (in addition to the acidity change) of great importance was found, regulating the calcium phosphate precipitation leading to ossification. ROBISON found that the enzyme had the greatest activity in ossifying cartilage, bones, and teeth of very young animals, the activity per unit weight of tissue decreasing with age. Although the plasma contains on an average only 0.5 mgm of phosphorus present as phosphoric ester per 100 cc. this is completely hydrolysable by the bone phosphatase and thus supplies phosphate ion amounting to about $^1/_6$ of the inorganic phosphorus present in the plasma, an amount amply sufficient to bring about a supersaturation and a subsequent precipitation of calcium phosphate, or more correctly of the apatite-like bone substance, from the already nearly or fully saturated plasma. The conclusions arrived at in this paper are independent of the special mechanism assumed for the ossification process.

DISTRIBUTION OF LABELLED PHOSPHORUS IN THE INCISORS OF RATS

The rapidly growing incisors of rats are very suitable for studying the distribution of phosphorus. According to FRIDERICA and GUDJONSONS[2] the average extrusive incisor growth per week is 2.7 mm. in the case of adults and 3.4 mm. for young rats. As seen in Fig. 1 A the cross section of the pulpa is very large at the proximal end and gets narrower toward the distal end, the last millimetres of the teeth being free of pulpa. The problem we have to investigate is how the distribution of newly formed calcium phosphate in the incisor takes place. Two extreme cases must be envisaged:

a) the labelled phosphate is deposited

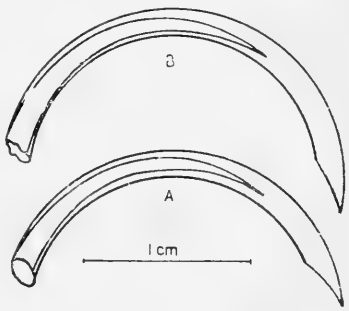

FIG. 1.

[1] R. ROBISON (1912) *The Significance of Phosphorus Esters in Metabolism*, New York.

[2] L. S. FRIDERICA and S. V. GUDJONSONS, *Kgl. Danske Vid. Selsk. Biol. Med.* **28**, 813 (1931).

in close proximity to the pulp from which it is derived, while the tissue formed at an earlier date is pushed along in the direction of growth;

b) the labelled phosphate is equally distributed throughout the incisor.

Cutting incisors tranversally into pieces and analysing these separately revealed the fact that the largest part of the labelled phosphate is found in those regions of the incisor where the pulpa is strongly developed, but that some of the labelled phosphate is found all through the incisoral tissue (Tables 5 and 6).

Table 5. Distribution of Labelled Phosphorus, Contained in the Normal Diet, Found in the Incisor after 2 days. Weight of the Rat 210 gm + Denotes Upper — Lower Teeth

Part of the incisor	Weight of ash in mgm	% of labelled P taken found	% of the labelled P per mgm ash
Proximal I + ...	38.2	0.42	0.011
Proximal II+	40.8	0.47	0.012
Proximal I ÷	29.2	0.37	0.013
Proximal II÷	27.2	0.38	0.014
Middle	92.6	0.125	0.00135
Distal I + ...	115.2	0.072	0.00063
Distal II÷	36.4	0.008	0.00022

Percentage of labelled P found in the total incisors = 1.85. Average per 1 mgm ash = 0.005. Biggest ratio between proximal and distal end = 60.

Table 6. Distribution of Labelled Phosphorus, Administered in the Normal Diet, found in the Incisor after 7 Days. Weight of the Rat 240 gm

Part of the incisor	Weight of ash in mgm	% of labelled P taken found	% of the labelled P per mgm ash
Proximal I + ...	25.0	0.28	0.011
Proximal II+	23.6	0.31	0.013
Proximal I ÷	21.8	0.29	0.013
Proximal II÷	31.6	0.32	0.010
Middle+	81.6	0.204	0.0032
Middle ÷	68.0	0.206	0.0031
Distal + and ÷	29.3	0.020	0.00074

Percentage of labelled P found in the total incisors = 1.69. Average percentage per 1 mgm ash = 0.006. Biggest ratio between proximal and distal part = 18.

In the experiments now to be described the distal part of the incisor was removed by operation one day before labelling the phosphorus present in the blood. In these experiments the radioactive P was not added to the food but given in the form of subcutaneous injections. 2 days, 5 days and 8 days after the administration of the labelled phosphorus the end part of the freshly grown incisor was again removed by operation and its radioactivity ascertained. The distal parts removed were all outside the range of the pulp. The figures obtained are seen in Table 7 and those from a similar experiment in Table 8.

TABLE 7.

Days after intake of labelled P	Weight of the tissue in mgm	% of the labelled P found in 1 mgm fresh tissue
2	42.8	0.00089
5	16.4	0.00030
8	20.4	0.00066
13 (rat killed)	26.6	0.00090

Percentage of the labelled P found in mgm of average incisor tissue = 0.0076. The removed distal ends contained 8 to 25 times less labelled P than the average tissue.

TABLE 8.

Days after intake of labelled P	Weight of the tissue in mgm	% of the labelled P found in 1 mgm fresh tissue
5	14.4	0.00040
8	11.0	0.00044
13 (rat killed)	21.3	0.00062

Percentage of the labelled P found in 1 mgm of average incisor tissue = 0.0062. The removed distal ends contained 10 to 16 times less labelled P than the average tissue.

Though the figures in the tables above clearly show that the deposition of labelled phosphorus is not restricted to the regions in the vicinity of the pulp, but that the labelled phosphorus is to be found even in the most remote part of the incisors, we attempted to obtain incisors with an appreciably larger pulp-free part. As is well known, rats, being rodents, grind their teeth and thus continually remove parts of the pulp-free end of the growing incisors. By eliminating the upper incisors the animal was prevented from gnawing and incisors were thus obtained in which the distal pulp-free end had a length of 10.5 mm. as shown

Table 9. Distribution in the Incisor after 3 days. Labelled Phosphorus Injected Subcutaneously. Weight of the Rat about 200 gm

Part of the incisor (comp. Fig. 2)	Weight of the tissue in mgm	Weight of the ash in mgm	% labelled P found per mgm tissue	% labelled P found per mgm ash
I (Proximal end)	13.1	9.2	0.0103	0.0151
II	14.5	11.0	0.0079	0.0116
III	24.0	17.1	0.0026	0.0040
IV	15.3	11.0	0.00021	0.00030
V (Distal end)	12.0	9.0	0.000033	0.000044

in Fig. 1 B. The result of this experiment is seen in Table 9 and the diagram Fig 2.

The content of labelled P varies between 0.01% at the proximal end and 0.000033% at the pulp-free distal end, thus diminishing by a factor

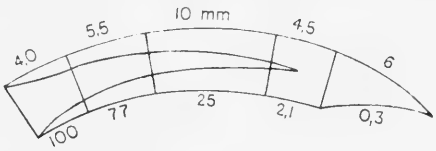

Fig. 2. Distribution of labelled phosphorus in the incisor of a rat killed 3 days after the administration of the phosphorus. The figures below give the relative amounts of labelled phosphorus present in 1 mgm of fresh tissue in the section in question. The figures above give the length of the section in mm

of 1/300. On comparing the activity of the ash obtained by igniting the incisor the figures work out to be 0.015% and 0.000044% respectively, corresponding to a factor of 1/340. The average content of labelled P in 1 mgm tissue was found to be 0.0041%, in 1 mgm ash 0.0059%. Fig. 2 shows both the location of the pulp and the distribution of the labelled phosphorus. It is seen clearly that the bulk of the labelled phosphorus atoms are to be found in the vicinity of the pulp but an amount which is far from being negligible reaches even the remotest part of the incisor. In Fig. 2 we have inserted the relative abundance figures of the labelled phosphorus present in the different parts of the incisor. A part of the first sector amounting to 1.4 mm. grew during the time which elapsed between the injection of the labelled P and the killing of the animal; the other parts were present before. Out of 204 parts of labelled phosphorus only 100 were found in the first sector and consequently not more than 30 in the part actually grown, the remaining 174 or more being at least partly located in the parts present before injecting the phosphorus.

In seeking an explanation of the presence of labelled phosphorus at a very considerable distance from the pulp we must remember that even the most remote incisal part of the tooth contains organic constituents. The constituents of the blood plasma penetrate through the latter and exchange of phosphate radicles and possibly also some ossification occurs *in situ*, though only to a modest extent on account of the poor circulation in comparison with that in the vicinity of the pulp. For part V (Fig. 2) we found a loss of weight on ignition amounting to 25% of the weight of the tissue dried in a vacuum dessicator; part I lost 29.8%; and the average loss in ignition was found to be 27.4%. That bones rich in organic constituents, i. e. such in which a comparatively effective circulation takes place, take up more radioactive phosphorus than the diaphysic bones poor in organic constituents had already been found previously in our investigations and is also to be seen in an example discussed on page 178.

PHOSPHORUS EXCHANGE IN GROWING RATS.

It is tempting to explain the parallelism between the abundance of organic substance present in the tissue investigated and the percentage of labelled phosphorus present by assuming that the latter is chiefly present in the organic substance and not in the calcium phosphate of the bone or teeth. This possibility must however be discarded because blood weighing as much as an incisor contains after the lapse of few days less than 0.01% of the labelled phosphorus taken, while that found in the incisors exceeds 1%. In view of the importance of this point we tested the effect of the removal of the pulp on the exchange data. The experiment was carried out on a young rat which increased in weight from 87 to 110 gm in the course of the 5 days which elapsed between the subcutaneous administration of the labelled phosphorus and the killing of the animal. Before the analysis the pulp was removed from the two upper incisors and the activity of these incisors compared with that of the two lower incisors containing the pulp: we also measured the activity of the extracted pulp. The results are seen in Table 10.

TABLE 10.

	Fresh weight	Ash weight	% of the labelled P found per mgm ash	Loss of weight on ignition
Lower Incisors + pulp	58.2	37.7	0.0192	35.2%
Upper Incisors − pulp	33.2	22.9	0.0185	31.0%
Tibia	800.1	123.2	0.0234	84.6%

The activity of the extracted pulp was very weak and only amounted to about 3% of that of the upper incisors. Should the exchange of phosphorus in the calcium phosphate of the teeth be very small, it is however quite possible that the amount of labelled phosphorus present in the pulp would no longer be negligible (comp. p. 26).

While in the experiments described above emphasis was laid on the investigation of the remote incisal end, in the following experiment we cut the proximal end of the incisor into small pieces and compared their activity with that of the distal end. The results are seen in Table 11, I denoting the united parts nearest to the jaw of all four incisors (comp. Fig. 3).

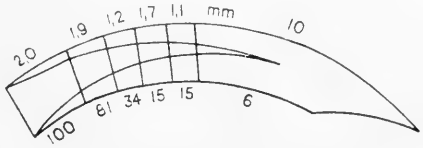

Fig. 3. Distribution of labelled phosphorus in the incisor of a rat killed 7 days after administration of the phosphorus.
The figures below give the relative amounts of labelled phosphorus present in 1 mgm of fresh tissue in the section in question. The figures above give the length of the section in mm. 2.0 should read 2.8.

Table 11. Distribution of Labelled Phosphorus, Injected Subcutaneously, In the Incisor after 7 days. Weight of the Rat about 200 gm

Part of the incisor (comp. Fig. 3)	Length in mm.	Weight of the fresh tissue in mgm	% of the labelled P found per mgm tissue
I	2.8	7.3	0.0156
II	1.9	16.8	0.0127
III	1.2	21.3	0.0054
IV	1.7	25.1	0.0025
V	1.1	28.2	0.0025
VI	10.0	163.7	0.00096

Average % of labelled P per mgm tissue = 0.0028, per mgm ash 0.0038.

The investigation of the labelled P content of the head (A), the central (B) and lower part (C) of the tibia gave the figures seen in Table 12.

While in the proximal end of the incisor the phosphorus exchange is much greater than in any part of the tibia, the exchange of the average phosphorus atoms in the tibia is about 29% greater than that of the average P atoms of the incisor; this is due to the fact that contrary to the tibia a large part of the incisor exchanges phosphorus atoms

TABLE 12.

	Weight of the fresh tissue in mgm	Weight of ash in mgm	% of the labelled P found per mgm ash
A	131.1	144.5	0.0080
B	312.4	130.9	0.0026
C	243.8	63.5	0.0027

in the course of 7 days only to a small extent. The figures in Table 12 cannot be compared directly with those obtained from fully grown animals for the following reason: the growing animal being much smaller the percentage of labelled P obtained for the same weight of the organ becomes larger; furthermore growth much facilitates the uptake of phosphorus. We can, however, compare the ratio of the labelled P content of the incisor and of other organs; the value of this ratio for the tibia, for example, is found to be not appreciably different in the cases discussed above. As to the labelled phosphorus content of the blood, this amounted after the animal was killed to only 0.04% per gram of blood; assuming a blood content of 10 cc. the circulation contained but 0.4% of the labelled phosphorus administered of 5 days.

THE EXCHANGE OF LABELLED PHOSPHORUS IN MOLARS

In contrast to the incisors, molars of adult rats do not grow, so the labelled phosphorus found in the latter is due solely to exchange processes; the blood stream circulating through the molar carries labelled phosphate ions which enter into exchange processes with the calcium phosphate of the molar tissue. Such exchange processes also take place in the incisors simultaneously with the formation of new ossification products. In the molars of adult animals, however, we encounter chiefly the former process; but though growth can be excluded we cannot discard the possibility of dissolution of tooth tissue at one place and a corresponding precipitation of calcium phosphate at another along the boundary between the circulating fluid and the tooth tissue. Small fluctuations in the acidity or parathormone concentration of the blood are sufficient to cause such a process. The molars of the rat decribed on p. 181 showed a content of labelled phosphorus amounting to 0.0013% per mgm of tissue and 0.0018% per mgm of ash, which is less than in the average incisor. The loss on ignition was found to be 26.9%. We thought furthermore that it would be of interest to compare the labelled P content of the incisors, molars and skeleton, choosing the tibia as representative of the latter. The figures obtained are seen in Table 13.

TABLE 13.

Organ	Labelled P found in 1 mgm fresh tissue as a percentage of the amount given	Labelled P found in 1 mgm ash as a percentage of the amount given	Loss in weight on ignition
Incisor	0.0033	0.0044	26.4%
Molar	0.0013	0.0018	26.9%
Tibia	0.0024	0.0064	63.2%

The fresh tissue of the incisors contains more labelled P than equal weights of either the molars or the tibia, but comparing the ashes the tibia has a larger labelled P content than both the incisors and the molars. A comparison of the labelled P content of the ash is in general preferable to that of the fresh tissue, the former comparison giving information about the percentage of phosphorus atoms replaced by labelled ones. The total P content of the ash of the incisors varies between 19.6 and 20.0%, and that of the molars and the tibia is only slightly smaller, about 18%. A closer analysis of the tibia revealed the parallelism already mentioned between the content of organic tissue and labelled phosphorus; that is shown in Table 14. The rat was killed 3 days after the administration of the labelled P.

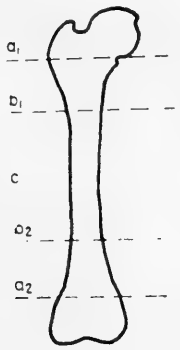

FIG. 4.

As seen from the figures of Tables 13 and 14 the head of the tibia has exchanged a part of its phosphorus content eight times as large as that exchanged by the molars.

Furthermore we compared the labelled P content of the incisors, the molars, and the tibia in the case of a rat weighing 220 gm killed 1 hour after the labelled phosphorus had been administered by subcutaneous injection. The results are seen in Table 15. The incisor was

TABLE 14.

Part of the tibia, see Fig. 4	Weight of the fresh tissue in mgm	% of the labelled P found in 1 mgm tissue	% of the labelled P found in 1 mgm ash	Loss on ignition
a_1	215.4	0.0028	0.0134	79.1
b_1	64.4	0.0033	0.0064	48.3
c	78.8	0.0019	0.0033	42.0
b_2	33.1	0.0015	0.0028	47.5
a_2	81.5	0.0014	0.0034	58.7

Table 15.

Organ	Labelled P found in 1 mgm fresh tissue as a percentage of the amount given	Labelled P found in 1 mgm ash as a percentage of the amount given	Loss in weight on ignition
Incisor	0.00046	0.00062	26.0
Molar	0.00025	0.00034	27.4
Jaw	0.00125	0.0020	36.3
Tibia head	0.0024	0.0077	68.7
Tibia residue	0.00068	0.0014	52.7

cut in 5 pieces the labelled P content of which is seen below, I denoting the proximal end.

Table 16.

	Weight of the fresh tissue in mgm	Labelled P found in 1 mgm fresh tissue as a percentage of the amount given
I	6.1	0.0062
II	14.4	0.0027
III	236.2	0.00040
IV	33.0	0
V	19.6	0

One cc. of blood contained 0.5% of the activity injected; assuming a blood content of 10 cc., only 5% of the labelled phosphorus injected was present in the circulation after the lapse of 1 hour. Only 1 hour after administering the labelled phosphorus the tibia phosphorus was found to be 1000 times less active than the blood phosphorus, while for the molars the corresponding ratio was found to be 5000 and for the incisors (inclusive of growth) 2700. We also determined the activity of the acid soluble phosphorus extracted from the muscles of the rat and found 1 mgm to contain 0.042% of the labelled P given. From this figure and those found for the activity of the tooth and tibia phosphorus to be seen in Table 16 it follows that a comparatively fast phosphorus exchange is taking place in the muscle compared with that ascertained in the bones and the teeth.

EXCHANGE OF PHOSPHORUS IN THE TEETH OF CATS

For the teeth of young cats[1] killed a few hours after the subcutaneous injection of the labelled phosphorus the results seen in Table 17 and 18 were obtained.

Table 17. Cat Weighing 2 kgm Killed after $3^1/_2$ Hours

Tooth	Weight of ash in mgm	% of injected labelled P present in the tooth	% of the labelled P per mgm ash of the tooth
Upper molar	123	0.016	0.00013
Lower molar	110	0.014	0.00013
Upper canine	108	0.044	0.00041
Lower canine	91	0.040	0.00044

Table 18. Cat Weighing 2.5 kgm., Killed after $1^1/_2$ Hours

Tooth	Weight of ash mgm	% of injected labelled P present in the tooth	% of the labelled P per mgm ash of the tooth
10 Incisors	94.8	0.0032	0.000034
Canine	148	0.019	0.00013
Jaw	126.3	—	0.00037

We also investigated fully grown cats. A cat weighing about 4.5 kgm and killed three days after administration of the labelled P gave the figures seen in Table 19. In this experiment the labelled P injected was not of negligible weight but amounted to 15 mgm (corresponding to about 75 mgm sodium phosphate). The labelled phosphorus used in this experiment was kindly presented to us by Prof. Lawrence and was prepared by the action of high speed deuterium ions on phosphorus and accordingly contained a comparatively large amount of normal phosphorus. The injection of 15 mgm P into a cat leads to an accelerated excretion and the figures are thus not entirely comparable with those of the last described experiment, which was furthermore carried out on a growing cat.

[1] The heads of the cats were kindly given to us by Professor Lundsgaard: they were obtained in the course of an investigation on the distribution of labelled phosphorus carried out by him and one of the present writers. In the first mentioned case 1 mgm plasma P was found to contain after $3^1/_2$ hours 1.6% of the activity injected, i. e. about 2300 times as much as that present in 1 mgm of the upper molar P.

Table 19. Cat Weighing 4.5 kgm., Killed after 3 Days.

	Weight of ash in mgm	% of injected labelled P present in the teeth	% of the labelled P per 100 mgm ash of the tooth
Molars	690.5	0.0080	0.0012
Upper canines	768.4	0.0076	0.0010
Lower canines	635.2	0.0068	0.0013

The corresponding enamels weighed 29.3, 34.3 and 55 mgm. The canine enamel was found to contain less than $^1/_{90}$ of the labelled P content of the corresponding dentine.

In another experiment a strong preparation was administered in three portions, 5 days, 2 days and 1 day before killing the animal, each portion containing 40 mgm P. The results are seen in Table 20.

Table 20. Cat Weighing 4 kgm., Killed after 5 Days.

	Weight of teeth in mgm	Weight of ash in mgm	% of injected labelled P present in the teeth	% of the labelled P per 100 mgm ash of the tooth
Molar	323.3	186.0	0.0027	0.0015
Canine	422.2	274.3	0.0038	0.0014
8 Incisors	172.5	117.5	0.0021	0.0018

The enamel obtained is discussed on page 183. In investigating the incisors of rats we found the activity to be due almost exclusively to the phosphate of the mineral constituents, the pulp being only slightly active. In the earlier experiments conditions were however very different from those obtaining in the above mentioned case. The uptake of labelled P in the teeth of a cat is much smaller than in the incisors of a rat and correspondingly the ratio of labelled P in the plasma to labelled P in the teeth is much larger in the case of the cat. Now a high blood activity will lead to a comparatively high pulp activity and we must expect a greater share of the pulp[1] in the total activity of the tooth in the case of cat teeth. To test this point we removed the pulp of some of the canine teeth and compared the activity of the dissected and the total canine. We found an activity ratio of 3 : 4, showing that a quarter of the activity of the canines of a fully grown cat is due to the pulp.

A comparison of the figures of Tables 17 and 18 with those of 19 and 20 shows that the uptake of labelled P in young animals is greater than

[1] Human tooth pulp was found by H. C. Hodge, *Proc. Soc. Exp. Biol. Med.* **35**, 53 (1936) to contain 0.70% phospolipins besides other phosphorus compounds.

in fully grown ones and also that while in the former case the canines take up 3 to 4 times as much labelled P (per mgm ash) as the molars, in the latter case no such difference is found. As has already been mentioned above the figures for the two sets of experiments are not entirely comparable, but no objection can be raised against a comparison of the ratio of the canine and molar uptake, which differs very markedly in the case of growing rats from the ratio for fully grown animals. The following is a possible explanation of this difference: the labelled P uptake in the teeth of young rats is due partly to a growth of the teeth and not to an exchange process; since in the cat the canines grow faster than the molars the uptake is greater in the former case. One would be inclined to object to this explanation in view of the short duration of the experiment, as the growth in the course of few hours may be entirely negligible. This objection is however unwarranted. The molars of the growing cat weighed 116 mgm and those of the fully grown animal 691 mgm. It does not take longer than a few years for the growing cat to become fully grown so the yearly growth of a molar will be above 100 mgm.

Let us now calculate the amount of tooth ash formed on the assumption that the labelled phosphorus found in the tooth is due to growth. A molar of the growing cat took up 0.016% labelled P during 3.5 hours. The labelled P which we injected into growing cats had in most cases a negligible weight originally, but very soon after the injection it mixed with the inorganic phosphate of the plasma (corresponding to about 5 mgm P) and from that moment we must consider the labelled P as having a weight of about 5 mgm 0.016% of the labelled P will therefore correspond to 0.0008 mgm P. The next step is that a large part of the labelled phosphorus leaves the plasma and is replaced by other phosphorus atoms coming from different bodily organs and also from the blood corpuscles. The result is that 0.016% of the activity given no longer represents 0.0008 mgm P but a greater weight, our scale of indication becoming less and less sensitive. From the experiences of Prof. Lundsgaard and one of us on the exchange of phosphorus present in the plasma we can estimate roughly that the amount of P which corresponds after the lapse of 3.5 hours to 0.016% of activity is about 0.008 mgm in the case discussed. To transform from phosphorus weight to ash weight we have to multiply by six. The weight of the tooth thus increases by 0.04 mgm in 3.5 hours and about 100 mgm in a year. The order of magnitude of the growth observed and that calculated on the assumption that the uptake of labelled P is due to growth is thus the same.

A very simple but instructive calculation can be carried out in the case of a fully grown cat into which as much as 120 mgm labelled P was injected. We can calculate how many milligrams of these 120 mgm

are to be found after the lapse of 5 days in a single tooth. Making use of the figures quoted in Table 20 we find that a canine takes up 0.005 mgm and a molar 0.003 mgm.

THE BEHAVIOUR OF THE ENAMEL

The difference in the mechanical properties of dentine and enamel is very pronounced. The hardness of anterior enamel is nearly half as great as that of hardened toolsteel, while dentine compares closely with brass[1]. The hardness is taken as the pressure in kilograms necessary to push a steel ball into the test piece.

The above mentioned difference is not due to a pronounced difference in the relative abundance of the mineral constituents of dentine and enamel, as discussed on p. 5, but to the following conditions. The amount of organic constituents+water found in dentine is about six times as large as the amount present in enamel, the calcification of the enamel tissue being thus carried through much more effectively than that of the dentine tissue. Bowes and Murray[2] found organic matter in human enamel to an extent of only 1%. As there is more organic matter[3] in enamel near the junction with the underlying tissue, the dentine, than in the part equidistant from the dentine and the surface of the teeth, the outer part of enamel must contain even less than 1% organic matter. The latter appears to be[4] a protein containing tyrosin and resembling reticulin.

Another outstanding difference between dentine and enamel seems to be the size and degree of orientation of the crystallites present in these. As to the orientation it has been stated[5] that enamel of high quality gives X-ray diagrams of a high degree of orientation, while enamel of poor quality does not. On igniting dentine an X-ray diagram characteristic of β-$Ca_3(PO_4)_2$ is often but not always observed[6]; this is never shown by ignited enamel. As it was found[7] that β-$Ca_3(PO_4)_2$ is formed when an excess of PO_4-ion is present, it was concluded that the dentine apatite often adsorbs an excess of phosphate ion which promotes the formation of β-$Ca_3(PO_4)_2$ on ignition. In the case of enamel forming larger crystallites, no excess of PO_4-ions being present, no β-$Ca_3(PO_4)_2$

[1] H. C. Hodge, *J. Dent. Res.* **15,** 251 (1936).
[2] J. H. Bowes and M. M. Murray, *Biochem, J.* **29,** 721 (1935).
[3] C. F. Bodecker, *J. Dent. Res.* **6,** 2, 117 (1923).
[4] P. Pincus, *Nature* **138,** 970 (1936).
[5] J. Thewlis, *Naturw.* **25,** 42 (1937).
[6] W. F. Bale, M. L. Lefevre and H. C. Hodge, *Naturw.* 24, 976 (1936).
[7] G. Trömmel and H. Möller, *Z. anorg. Chem.* **206,** 227 (1932).

formation was observed on ignition. While important information may be obtained by the study of X-ray diagrams the interpretation of the latter must be made with care.

PHOSPHORUS EXCHANGE IN THE ENAMEL

In view of the connection found between the content of organic matter and phosphorus exchange in the teeth it did not appear very promising to look for a pronounced exchange in the enamel. The enamel investigated by us was in some cases removed mechanically while in others we succeeded in separating the enamel of cat teeth after igniting the tooth very carefully. The enamel, having a different expansion coefficient from the dentine, splits off during the ignition process and can thus be removed. The method of separation used recently by various workers[1], in which the tooth is pulverized and placed in an organic liquid of suitable density when the heavier enamel settles to the bottom of the tube, is not suitable for our purpose. The reason is that some dentine often sticks on the pulverized enamel; assuming that the dentine is strongly active and the enamel not, we see that the presence of traces of dentine in the enamel might falsify the analysis.

We made several experiments with the enamel of cat teeth but in most cases with negative results, the exchange in equal weights of enamel being at least 20 times as small as that found in the molars of cats. In one case we got a positive effect, the canine of a fully grown cat five days after injecting the labelled phosphorus showing a radioactivity of 26 relative units (counts per minute), one enamel sample showing 0.6, and another 0.7 counts. The first mentioned enamel was separated by grinding it off from the dentine, while the second one was obtained by the same method from the uppermost enamel layer. The ash weight of the canine was 277.3 and that of the enamel samples 33.1 and 19.1 mgm. We are however reluctant to accept this positive result. On account of its smaller weight and greater distance from the underlying dentine, the outermost layer should be less active than the second enamel layer, unless the labelled phosphorus present in the saliva (which 13.4 mgm. % P 100 cc.) can interact with the outer layer of the enamel. We intend to follow up the problem of the phosphorus exchange in enamel using phosphorus preparations of greater activity.

[1] Comp. P. J. Brekhus and W. O. Armstrong, *J. Dent. Res.* **15,** 23 (1935).
[1] M. Karshan, *J. Dent. Res.* **15,** 388 (1936).

EXCHANGE OF PHOSPHORUS IN HUMAN TEETH

Other things being equal the exchange of phosphorus in teeth will be determined by the efficiency of lymph circulation in the tooth. Exchange experiments can thus be carried out to obtain information on the latter point. It does not look improbable that the growth of caries will be facilitated by a poor circulation; to decide this point we compared the phosphorus exchange in two teeth of the same individual (16 years old) removed simultaneously, one on account of caries, the other, a healthy one, to space the patients teeth better; about a two hundred thousandth part of the labelled phosphorus was found in each of the teeth investigated, a quantity sufficient to be measured but not large enough to permit the exact comparison necessary to decide the point discussed above. The weights of the whole fresh teeth were 800 and 540 mgm and of the ash obtained on ignition 465 and 330 mgm this corresponds to a loss on ignition of 58 and 61%. The time which elapsed between the injection of the radioactive phosphorus and the extraction of the teeth was 7 days. Through the very great kindness of Professor LAWRENCE we were able to continue these experiments using a much stronger radioactive phosphorus sample

TABLE 21.

LABELLED PHOSPHORUS IN THE TEETH OF A 25 YEAR OLD PATIENT

a) Necrotic Roots.

	Fresh weight	Ash weight	Relative labelled P content	
			In total root	In 100 mgm root ash
1	223.7	138.1	2.7	1.96
2	284.1	190.1	4.9	2.72
3	199.4	127.1	2.5	1.97
4	230.5	143.0	1.4	0.98
5	124.8	76.5	3.9	5.13
6	435.2	268.5	5.9	2.19
7	205.7	127.1	4.9	3.86
8	169.5	109	1.6	1.47
9	172.5	106.6	3.8	3.57
10	183.5	115.0	2.1	1.83

prepared by him with the aid of his powerful cyclotron. 900 mgm labelled sodium phosphate per os were administered to a patient 25 years old. 4 days later 10 necrotic teeth and 5 days later still, three more, fairly well preserved, living teeth were extracted. Of the $2.5 \cdot 10^6$

relative radioactive units we can estimate that about $1.8 \cdot 10^6$ were absorbed. As is seen in Table 21/6 relative units were found in a fairly well preserved tooth on an average, showing that about 1 : 300,000 part of the labelled phosphorus atoms enter a single tooth; in the case

b) Necrotic Crowns

	Fresh weight	Ash weight	Relative labelled P content	
			In total crown	In 100 mgm crown ash
One single crown	65.8	39.1	3.7	9.4
Fragments of several crowns	241.7	149.8	12.3	8.2

c) Almost normal roots

Nr.	Fresh weight	Ash weight	Relative labelled P content	
			In total root	In 100 mgm root ash
1	377.4	241.0	2.8	1.16
2	651.1	413.6	3.7	0.9
3	685.9	430.2	6.7	1.56

d) Almost normal crowns

Nr.	Fresh weight	Ash weight	Relative labelled P content	
			In total crown	In 10 mgm crown ash
1	533.5	338.7	1.9	0.56
2	862.9	670.9	1.8	0.27
3	464.1	429.9	1.8	0.42

of a 16 years old boy about 1 : 200 000 was found. In the latter case an activity of only 0.5 units (counts per minute) was shown by a single tooth, and the estimate was accordingly only a very rough one. From the above result it follows that about 1 : 300,000 part of the phosphorus taken up with the food finds its way into each tooth of an adult.

Summary

It has been shown that an exchange of phosphorus atoms present in the teeth with those present in the blood plasma takes place.

During the growth of the incisors of rats the newly deposited phosphorus atoms are to a large extent found in close vicinity of the dental pulp, but even in the most remote part of the incisor presence of newly substituted phosphorus atoms can be established. An exchange of phosphorus atoms thus takes place even in those parts of the incisors which are entirely outside the range of the pulp. The exchange in the molars was found to be less pronunced than that in the incisors, this being presumably due to the fact that these do not grow.

In the teeth of young cats within fews hours., besides an exchange of phosphorus atoms, an increase in the labelled phosphorus content due to the growth of the teeth could already be ascertained.

An exchange of phosphorus has also been proved for human teeth, 1:300,000 of the phosphorus administered being found in each tooth. The replacement of 1% of the phosphorus content of a human tooth by phosphorus atoms taken up with the food takes about 250 days.

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21. RATE OF REJUVENATION OF THE SKELETON

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THE first experiments in which labelled (radioactive) phosphorus ^{32}P was applied as an indicator [CHIEVITZ and HEVESY, 1935], showed that some of the phosphorus atoms of the mineral constituents of the bone exchange rapidly with those present in the plasma. This result was corroborated and extended by later work on this subject [HEVESY *et al.*, 1937; COOK *et al.*, 1937; DOLS *et al.*, 1937; 1939; ARTOM *et al.*, 1938; COHN and GREENBERG, 1939; LEFEVRE and BALE, 1939]. The question as to what extent the P contents of the mineral constituents of the bone are replaced in a given time by plasma P remained unanswered however. This is an important question, as the rate of this replacement is a measure of the rate of rejuvenation of bone tissue. The extent to which bone P is replaced by plasma P in the course of a given time can be determined by comparing the activity of 1 mgm of bone P with that of 1 mgm of inorganic plasma P. The activity of the plasma P, i.e. its ^{32}P content, changes appreciably, however, with time. The ^{32}P atoms, like all P atoms present at any moment in the plasma, exchange with the P atoms present in the various organs and in doing so are removed from the plasma and replaced by tissue P. The application of the above-mentioned consideration implies a constancy of the activity of the plasma inorganic P. To secure such a constancy, we administered labelled phosphate all through the experiment, instead of doing so at the start of the experiment, as in all investigations mentioned above. By taking blood samples at intervals, we ascertained that the activity of the inorganic P of the plasma remained constant. At the end of the experiment, the bone sample was purified from all non-mineral constituents and the radioactivity of 1 mgm of bone P compared with that of 1 mgm of plasma inorganic P.

EXPERIMENTS WITH FROGS

Experiments of 5 to 240 min duration were carried out with frogs. As early as 5 min after injecting 0.3 ml. physiological NaCl solution containing a negligible proportion of labelled sodium phosphate into the lymph sac, the mineral

TABLE 1. LABELLED P CONTENTS OF PLASMA AND TIBIA OF A FROG

Wt. 45 gm: temperature 22°; Determinations 5 min after the start of the experiment

Fraction	^{32}P content per mgm P (specific activity)
Plasma	100
Epiphysis	0.026
Diaphysis	0.013

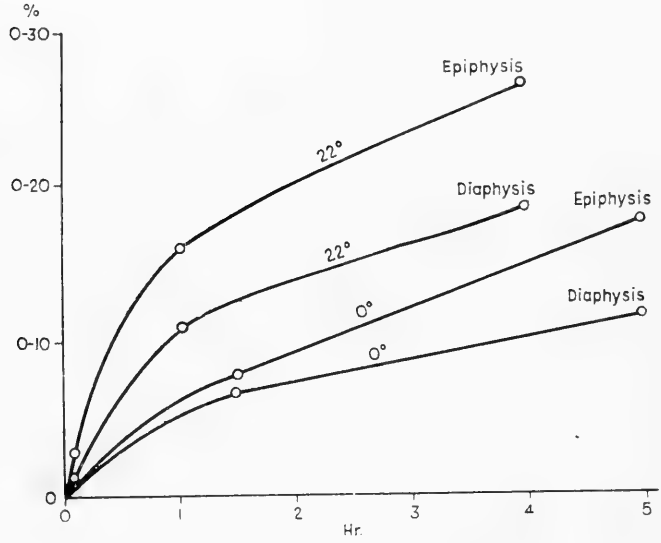

FIG. 1. Extent of replacement of frog's bone P by labelled P

constituents of the tibia contained some labelled phosphate, as shown in Table 1.

With increasing time, the ^{32}P content of the mineral constituents of the tibia increases (Fig. 1). After the lapse of 1 hr., the specific activity of the epiphysis P amounts only to 1/600 of the corresponding magnitude of the inorganic P of the plasma. Thus only 1/600 or less of the epiphysial P was replaced by plasma P within 1 hr. In the next 3 hr. a further 1/900 part of the epiphysis P exchanged. The first point in the curve was obtained by analysing the right, the second point by analysing the left tibia. For the diaphysis, the corresponding figures were found to be 1/900 and 1/1200, respectively. In the course of 4 hr., therefore, only a minute part of the tibia P is replaced by plasma P. A still smaller replacement is found when the frog is kept at 0°.

EXPERIMENTS WITH RABBITS

After the lapse of 2 hr., 1/530 and 1/1800, respectively, of the tibia epiphysis P and diaphysis P were found to be replaced by plasma P. With increasing time, an increase of the replacement of the bone P takes place, as shown in Fig. 2 and Table 2; this increase diminishes, however, with increasing time, as would be excepted. The bone tissue contains numerous small crystals formed by mineralization of the matrix. The crystals are built up on similar lines to the mineral apatite.[1] While the atoms situated on the uppermost layer of the crystals [Paneth, 1922] exchange easily with those present in the surrounding liquid, those situated inside the crystal are prevented from doing so, except at very high temperatures. The exchange between bone phosphate and plasma phosphate which we observe in experiments of short duration is due to a replacement process between the phosphate ions situated on the surface of the apatite crystals and those of the plasma or lymph. Should the surface exchange be exhausted, an increase of the time of the experiment may at first have no effect at all on the extent of replacement. If, however, in the course of time a dissolution and reprecipitation of the apatite crystals takes place, new and far-reaching possibilities

Table 2. Extent of Replacement of the Bone P of the Rabbit by Labelled P

The labelled phosphate was injected intravenously during the experiment

Fraction	2 hr. %	4 hr. %
Epiphysis	0.180	0.200
Diaphysis	0.056	0.106

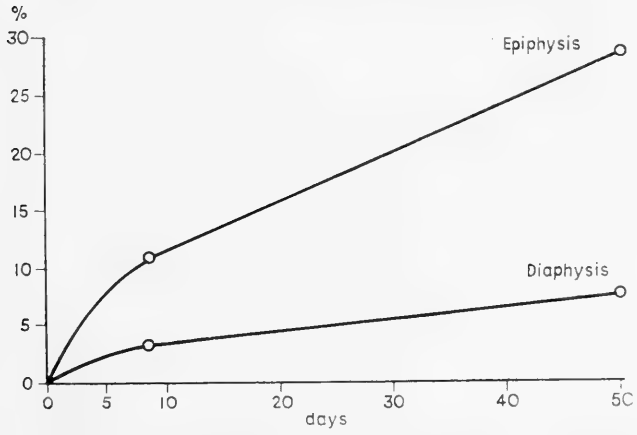

Fig. 2. Extent of replacement of rabbit's bone P by labelled P

[1] From X-ray measurement, it was concluded [Caglioti, 1936] that the inorganic part of the bone has the composition of about $3Ca_3(PO_4)_2 \cdot CaCO_3 \cdot xH_2O$ with the hexagonal structure of hydroxyapatite, the length of the axes being $a = 9{\cdot}2 \times 10^{-8}$ cm. and $c = 6{\cdot}9 \times 10^{-8}$ cm, the axis being oriented parallel to the length of the bone. The organic parts consist of polypeptide chains, supported and stretched.

of an exchange between plasma P and bone P will arise. From these considerations it follows that an exchange taking place within a long interval cannot be extrapolated from results obtained in experiments of short duration. We have, therefore, carried out experiments in which we kept the activity of the plasma inorganic P of rabbits at a constant level for several days or weeks.

EXPERIMENT OF LONG DURATION

To obtain a constant level of the plasma inorganic P, the first day every 30 min and later twice every day, labelled sodium phosphate of negligible weight was administered by subcutaneous injection to rabbits. After removal of the marrow, the bone was first extracted for 12 hr. with hot ether-alcohol. The bone was then treated with hot alkaline glycerol solution for further 6 hr. The fractions obtained were dissolved in HNO_3 and their P contents precipitated as molybdate. The molybdate was dissolved in dilute NH_3 and precipitated as ammonium magnesium salt. An aliquot of the sample obtained was used in the colorimetric P determination, while another aliquot was reprecipitated as ammonium magnesium phosphate and its radioactivity measured with a Geiger counter. In prolonged experiments the analysis of the plasma inorganic P is conveniently replaced by that of the urine P. In Tables 3 and 4 the specific activities of the different bone P fractions are recorded.

Table 3. Extent of Rejuvenation of the Tibia in the Course of 9 Days

Wt. of rabbit: 2 kgm

Fraction	% P rejuvenated (specific activity)
Epiphysis P	11.2
Diaphysis P	3.2
Tibia phosphatide P ...	74.8
Marrow phosphatide P .	80.1

In the course of 9 days therefore, only 11% of the epiphysis and 3% of the diaphysis are rejuvenated, while most of the phosphatide molecules present in the marrow and in the bone are newly formed.

In the course of 50 days, only 29% of the epiphysial and 7% of the diaphysial mineral constituents were replaced (Table 4). The tibia and femur show about the same behaviour. About half of the scapula remained unchanged. The almost complete replacement of the apical and medial parts of the incisor dentine P can hardly be interpreted as due to an exchange between dentine P and plasma P, since the replacement rate of the dentine P was found to be lower than that of the tibia P [Hevesy *et al.*, 1937; Lefevre and Bale, 1939] and since the tibia P, as seen above, was replaced only to a restricted extent. The high ^{32}P content of the incisor dentine must be due to an actual growth, to a formation by a calcification process. As a plasma containing ^{32}P was instrumental in calcifying the newly grown parts of the incisor, the P of the latter was bound to have the same specific activity as shown by the plasma P. As seen in Table 4, the P of the apical part of the incisor dentine investigated has, within the errors of experiment ($+5\%$).

Table 4. Extent of Rejuvenation of the Skeleton of a Rabbit in the Course of 50 Days

Fraction	% Prejuvenated
Femur epiphysis inorganic P	29.7
Femur epiphysis phosphatide P	100
Femur epiphysis glycerol extract* P	51.0
Femur diaphysis inorganic P	6.7
Femur diaphysis phosphatide P	*ca.* 100
Femur diaphysis glycerol extract* P	84.5
Tibia epiphysis inorganic P	28.6
Tibia diaphysis inorganic P	7.6
Costa	27.5
Scapula	43.8
Incisor dentine, apical	103
Incisor dentine, medial	98.5
Incisor dentine, incisal	41.2
Incisor enamel, apical + medial	82.0
Incisor enamel, incisal	6.6

* This fraction presumably contains some mineral P.

he same specific activity as that of the plasma P. This part, having a length of about 0.9 mm., is entirely newly formed during the experiment. The bulk of the medial part of the dentine was freshly grown as well, while the incisal part, having a length of 1.2 mm., is only partially newly formed with participation of the labelled plasma. About half of the P atoms present in the incisal part of the dentine were not labelled; they must thus be those which were located in the apical or medial region of the incisor before the start of the experiment. The tissue containing these atoms was pushed forward *in toto*. Partly before this "slipping" process and partly during it, some of the P atoms of the dentine have the opportunity to exchange with labelled P atoms and, therefore, the P of the incisal part of the dentine shows an activity which amounts to about 1/3 of the specific activity of the plasma P. We see here an interesting case of tissue formation in which macroscopic aggregates are "slipped" from one place to another *in toto*, experiencing only a restricted atomic or molecular replacement. This effect is much more clearly shown in the growth of the enamel.

The apical and medial parts of the enamel are formed by a calcification process from labelled plasma and, therefore, these parts of the enamel became strongly active. From the fact that the incisal part of the enamel is only slightly active, we have to conclude that this fraction is not formed in the course of the experiment through a calcification process. Its crystals were formed at an earlier date from non-labelled plasma and the whole fraction "slipped" *in toto* during the course of the experiment from the position in which it was calcified into the place it took up at the end of the experiment. The incisal end of the dentine is probably to a large extent also formed by "slip" *in toto* of the medial parts, though this conclusion is not supported as clearly by the activity figures arrived at in the case of the enamel. A part of the incisal dentine P had an opportunity to exchange to an appreciable extent before the "slip" took place and also during that process. Enamel P exchanges only to a minute extent [Armstrong, 1940]. It is also of interest to note that the activity figures exclude the possibility that

the incisal part of the enamel is formed by extensive calcification of the outer region of the dentine. In that case, the enamel could not be much less active than the corresponding dentine part. The foundation of the incisal enamel is laid in the apical end and reaches its final position without interchange with the dentine. As both the P and Ca of the teeth have their origin in the plasma, the application of labelled Ca as an indicator can be expected to lead to similar results to those found above.

EPIPHYSIAL AND DIAPHYSIAL BONE TISSUES

The epiphysis was found to exchange the P content of its mineral constituents at a higher rate than the diaphysis, as seen in Table 5.

TABLE 5. RATIO OF THE SPECIFIC ACTIVITIES OF THE EPIPHYSIAL AND DIAPHYSIAL P OF THE TIBIA OF RABBITS

The level of the activity of the plasma inorganic P was kept constant all through the experiment

Time	Ratio
2 hr.	3.2
4 „	1.9
10 days	3.5
50 „	3.8

In experiments with frogs, in which the labelled phosphate was injected into the lymph sac at the start of experiments, taking 1—22 days, the ratio 1.3—1.6 was found. In these experiments, the tibia and femur were both investigated and the average was taken. In an investigation of the tibia P of rats, to which the labelled phosphate was administered at the start of the experiment, the ratios 3.1, 2.9, 2.5, 1.7 and 1.8 were found after $\frac{1}{2}$ hr., 4, 10, 50 and 110 days, respectively. It is of interest to remark that from the finding that the diaphysial P is only about half as active as the epiphysial P 110 days after the start of the experiment, we can conclude that an appreciable part of the skeleton has not been renewed within 110 days.

In experiments on chickens, DOLS *et al.* [1939] found the above ratio to be 3, 22 hr. after administration of labelled phosphate; rachitic chickens gave the ratio 2.5.

The more rapid exchange of the epiphysial P is just what would be expected. The epiphysis is characterized by a poorer mineralization of the matrix than is the diaphysis and contains more organic matter and water than the diaphysis. The circulating lymph, containing the labelled P, will therefore reach the apatite surface more easily in the first-mentioned case. Should the size of the apatite crystals be smaller in the epiphysis than in the diaphysis and therefore the ratio surface: volume be larger in this type of bone tissue, one would also expect a more rapid exchange of the mineral constituents of the epiphysis. Whether such a difference in the size of the apatite crystals actually occurs is not known. X-ray investigations [BALE *et al.* 1934] lead to the result that the size of the ultimate

crystals of dentine and bone is about 10^{-6} cm.; while the very effectively mineralized enamel contains larger crystals (10^{-5} cm.). It is, therefore, quite probable that the difference in the extents of mineralization of the epiphysis and diaphysis manifests itself in a moderate difference in the sizes of the ultimate crystals in the two types of bone tissue. It is of interest to note that the difference in hardness of the different types of bones is, to a large extent, the result of a different degree of orientation of the crystallites of the bone. X-ray patterns indicate that orientation occurs during growth and first in those bones where the need for solidity (e.g. leg bones) is greatest [Caglioti and Gigante, 1936].

Summary

Labelled (radioactive) phosphate was administered to rabbits and frogs repeatedly during the experiment in order to keep the radioactive plasma inorganic phosphorus at a constant activity level. The comparison of the activity of 1 mgm bone inorganic P with that of the plasma inorganic P permits us to conclude to what extent the mineral constituents of the bone were renewed during the experiment.

Within 50 days, 30% of the femur and tibia epiphysis were found to be renewed, while the corresponding figure for the diaphysis amounted to 7%. Half of the mineral constituents of the scapula were found to be unchanged. The phosphatides of the bones and the marrow were entirely renewed.

The phosphorus of the apical part of the dentine of the rabbit's incisor was found to have the same activity as the plasma phosphorus. From this result it follows that this part of the dentine was grown with the participation of labelled plasma phosphorus during the experiment. The greater part of the incisal end of the dentine was not formed by a calcification process *in situ* but by a "slip" of the apical part of the dentine.

The same behaviour is shown even more pronouncedly by the enamel. No interaction of any significance takes place between the incisal part of the enamel and the dentine either during their formation or at a later date. The atoms present in the former are to a very large extent those which were previously located in the apical (medial) part of the enamel.

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22. RETENTION OF ATOMS OF MATERNAL ORIGIN IN THE ADULT WHITE MOUSE

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What percentage of the atoms present in the new-born organism is retained during the later phases of life and what percentage is inherited by the subsequent generations? An attempt was made to answer these questions by following the fate of the phosphorus atoms in the white mouse, radio-phosphorus being used as an indicator.

The phosphorus atoms present as constituents of different compounds in the body of the new-born animal are released successively from the compounds in which they are present. The phosphorus atoms thus released are either excreted or re-incorporated into various compounds present in the body, the latter process being much more frequent. Although, for the sake of simplicity, we speak of phosphorus atoms, practically no phosphorus atoms but only phosphate radicals are released from and built into such phosphorus compounds. The organism is supplied with phosphorus in the form of phosphate radicals and the phosphorus atoms adhere, as far as is known, to their partners throughout the numerous metabolic processes in which they participate.

The white mice used in the experiment were kept on the following diet. Wheat flour, oatmeal and a small amount of milk were administered, while on alternate days only Cooley's standard food was provided. Once a week, cabbage or lettuce was administered as well.

About 0.1 mgm of labelled sodium phosphate with an activity of a few microcuries was administered by subcutaneous injection to a pregnant mouse. As a result of introducing radiophosphorus into the organism of the pregnant mouse, we obtain offspring of which the phosphorus contents were labelled. A litter consisted of about 8 offspring having almost the same weight, as shown in Table 1.

The radio-phosphorus contents of the offspring may therefore also be expected to be almost equal. This fact makes it possible to determine the total ^{32}P content of the offspring at any date by measuring the total activity of any member of the litter.

One offspring was killed shortly after birth and dissolved in concentrated nitric acid. The phosphorus content of a known aliquot of the solution

was precipitated as magnesium ammonium phosphate. The precipitate was filtered through an aluminium dish of 1.1 cm diameter having a perforated bottom covered with filter paper. The dish containing the precipitate was then placed unter the Geiger counter. By comparing

Table 1. — Weight of 6 New-born Offspring of a Mouse

No.	Weight in gm	Relative activity
1	1.3	100
2	1.3	98.5
3	1.1	99.0
4	1.2	93.5
5	1.3	99.5
6	1.3	—

the activity of an offspring killed at a given date with the activity of another killed at a later date, it was possible to calculate what percentage of the phosphorus atoms of maternal origin was lost in the interval between the two dates.

All offspring were killed successively, dissolved, and treated in the way described above. The writer is much indebted to Mr. K. Zerahn for dissolving the mice and precipitating their phosphorus content. All offspring were killed and investigated within about three months. After the lapse of this time, the activity of the phosphate precipitates had decreased so greatly that it could no longer be measured with sufficient accuracy. The activity of the first offspring was compared with that of the second, the activity of the second with that of the third, and so on.

The mouse obtains its ^{32}P content not only by birth but also by lactation. In order to simplify the problem, to reduce the ^{32}P content of the offspring mainly to such ^{32}P as was obtained by birth, the active mother was replaced by an inactive mouse soon after gestation. As the replacement of the mother was not made immediately after the birth of the offspring, we actually measured the loss of ^{32}P acquired by birth plus the ^{32}P acquired by lactation in the interval between birth and replacement of the active mother by an inactive one, i.e. within a few days.

RESULTS

The result obtained are shown in the following tables.

TABLE 2. — MOTHER INJECTED FEBRUARY 9. DATE OF GESTATION: FEBRUARY 18. REPLACEMENT OF THE ACTIVE BY AN INACTIVE MOTHER: FEBRUARY 22. (EXPERIMENT 1.)

No. of offspring	Killed	Relative activity	Weight in gm
1	22/2	100	3
2	3/3	82	7
3	16/3	73	15
4	30/3	48	18
5	13/4	41	25
6	13/5	40	23

Loss of ^{32}P in the course of 81 days: 60 per cent.

In about 3 months,[1] a time sufficient for mice to reach adulthood,

TABLE 3. — MOTHER INJECTED FEBRUARY 9. DATE OF GESTATION: FEBRUARY 16. REPLACEMENT OF THE ACTIVE BY AN INACTIVE MOTHER: FEBRUARY 23. (EXPERIMENT 3.)

No. of offspring	Killed	Relative activity	Weight in gm
1	24/2	100	2.8
2	5/3	90	6.9
3	19/3	70	13
4	2/4	57	15
5	16/4	53	20
6	18/5	51	16
7	7/6	39	22

Loss of ^{32}P in the course of 103 days: 61 per cent.

only about 60 per cent of the ^{32}P content of the mouse acquired by birth is thus eliminated. The phosphorus content of the new-born mouse is found to be about 4 mgm the amount of P excreted by the mouse in the

[1] If only the last two values in Tables 2 and 3 are considered the average loss of ^{32}P in 80 days works out at 57 per cent. If all mice killed in April, May and June are considered, the average loss is 56 per cent in 72 days.

course of three month about 1 gm The fact that nearly one half of the maternal phosphorus is retained in the body, in spite of the comparatively large amounts of phosphorus excreted by the mouse in the course of 3 months, is due mainly to the protection of a large proportion of the phosphorus of the bones against interchange with the phosphorus atoms in circulation. The uppermost atomic layers of the bone apatite crystals interchange easily with the phosphorus atoms of the plasma or the lymph; furthermore, a kind of biological "recrystallization" takes place, i.e. dissolution of some molecular apatite layers followed by new formation of such layers through crystallization. All these processes, however, do not affect, or affect only at a very slow rate, large parts of the bone apatite which thus retain their P atoms. During the formation of the skeleton, a large proportion of the ^{32}P atoms present in the organism will find their way into the bone apatite and be fixed there to a very appreciable extent during the time of the experiment (3 months) or even for the lifetime of the mouse.

When comparing the ^{32}P content of several rats injected simultaneously with labelled phosphate at different dates, it was found (HEVESY 1939) that the ^{32}P, in so far as it was not excreted, accumulated to a very large extent in the skeleton. This fact is illustrated by the following table.

TABLE 4. — PERCENTAGE ^{32}P PRESENT IN THE BODY FOUND IN SOME ORGANS OF THE RAT

Organ	Time after which the rat was killed						
	½ hour	4 hours	10 days	20 days	30 days	50 days	98 days
Muscles	18.3	19.4	25.8	28.8	25.2	12.1	3.6
Carcass after removal of bones, muscles, blood and skin	37.0	13.6	21.0	16.4	13.1	6.7	2.9
Bones	19.1	23.4	13.1	13.1	51.8	76.5	92.6

Furthermore, when comparing the specific activity (activity of 1 mgm P) of the bone P with the specific activity of the plasma P of the rabbit in experiments where the activity of the plasma was kept at a constant level throughout, it was found (HEVESY *et al.*, 1940—2) that 70 per cent of the epiphysial P and 93 per cent of the diaphysial P of the tibia remained unchanged after 50 days. These facts illustrate the ability of the skeleton to prevent an interchange of a large part of its P atoms with the P atoms of the plasma, thus preventing an ultimate excretion of such atoms.

As the calcium atoms of the organism are found to a still higher percentage in the skeleton than the phosphorus atoms, the organism may be expected to retain the average calcium atom obtained by birth

still more jealously than it does the maternal P atoms. Similar considerations may apply to the magnesium and fluorine content of the organism. The atoms of maternal origin of all other elements present in the organism, however, can to be expected to leave the body at a much higher rate than do the phosphorus atoms of maternal origin.

THE PERCENTAGE OF ^{32}P TRANSFERRED FROM ONE GENERATION TO ANOTHER

Some experiments were carried out in order to follow the fate of the ^{32}P administered to a mouse in the second and the third generation.

The phosphorus content[1] of the new-born mouse constitutes about 2.4 per cent of the mother's phosphorus content; consequently, we should expect to find about 2.4 per cent of the ^{32}P contained in the pregnant mother in each new-born mouse. Actually, only about $^1/_4$ of that amount is found when comparing the activity of a mouse of the third and the second generation. This finding is explained mainly by the fact that a large part of the ^{32}P content of the mother is to be found in the skeleton and, as a large part of this ^{32}P does not reach the circulation, it does not participate in the formation of the foetus. The foetus acquires its phosphorus content mainly from the food phosphorus and the phosphorus present in the soft tissues. The phosphorus present in the circulation has a much smaller ^{32}P content than the average phosphorus of the mother, a fact which results in a comparatively low ^{32}P content of the new-born mouse. The first generation contains labelled phosphorus mainly in the soft parts of the body and in a minor part of the skeleton, and this because it obtained its phosphorus content by subcutaneous injection and not by the much more intimate foetal processes. The ^{32}P content of the first generation is therefore not strictly comparable with that of the second generation. The ^{32}P contents of the second and the third generations may, however, be compared. We may expect this ratio to be equal to that which we would obtain on comparing the ^{32}P contents of mice of the third and fourth or of the fourth and fifth generations etc. since it is to be expected that the percentage of ^{32}P passed from the third to the fourth generation will be the same as that transferred from the second to the third generation, etc. This conclusion is important since it is almost impossible to follow the fate of ^{32}P through more than three generations of mice.

From the injection of labelled phosphate to a mouse in the last stage of pregnancy (first generation) to the birth of the fourth generation

[1] The P content of a new-born mouse weighing 1.33 gm average value amounts to 4.2 mgm while that of a mouse weighing 31.8 g to 177 mgm

about 188 days elapse; this corresponds to 13 half-life periods of radio-phosphorus. During this time, the activity administered to the first mouse has decreased to less than 1/8000 of its original value. As the fourth generation may be expected to contain about 10^{-7} of the ^{32}P present in the first generation (apart from the radioactive decay), we may expect to find only about 10^{-11} of the radio-phosphorus administered to the first generation. The measurement of such a low percentage of radio-phosphorus administered would require the administration of 100 millicuries ^{32}P or more.

The phosphorus content of an adult mouse amounts to about 200 mgm or to $4 \cdot 10^{21}$ atoms. As of the ^{32}P atoms present in a mouse only ½ per cent are found in a mouse of the next generation, it is easy to show that the eleventh generation will no longer contain a single ^{32}P atom and thus no P atom at all which was present in the first generation.

This result illustrates strikingly the fact that the hereditary dispositions are entirely independent of any atomic community with the forefathers, these dispositions being determined exclusively by the faculty of the atoms and molecules to enter certain characteristic configurations. It is the pattern which matters and not the single brick.

Summary

Labelled phosphate was administered to a pregnant mouse. Each offspring was found to contain almost the same amount of ^{32}P. By killing the various offspring at different dates and comparing their ^{32}P contents, we determined the amount of maternal phosphorus atoms present in offspring at different times.

Between birth and maturity, i.e. in the course of three months, the mouse lost about 60 per cent of the P atoms acquired by birth.

By breeding three generations of mice, to the first generation of which ^{32}P was administered, the passage of phosphorus atoms from one generation to the next was followed. 0.6 per cent of the ^{32}P present at the birth of an offspring of the second generation was found to be present in an offspring of the third generation. We might expect to obtain the same ratio between the ^{32}P content of the third and fourth generation, and so on.

Making this assumption it can be shown that a mouse of the eleventh generation no longer contains a single phosphorus atom present in the first generation.

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23. RATE OF RENEWAL OF THE FISH SKELETON

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The phosphorus atoms present in the organism migrate from molecule to molecule and from organ to organ. The rate of migration greatly depends upon the nature of the molecules involved and on the organ in which they are located, the phosphorus atoms finding their most lasting abode in the skeleton. The mineral constituents of the skeleton are located in apatitelike crystallites which have a size of about 10^{-6} cm. From the phosphate ions present in these crystallites, only those located in the uppermost molecular layer can come into direct contact with the lymp or the plasma and, thus, participate in an interchange with the phosphate present in the plasma (lymph). The replacement of the bulk of the phosphate or other ions present in the apatitelike crystals can only take place by a partial or total dissolution of the crystallite followed by a crystallization process leading to a partial or total formation of new crystallites. This process is made possible by the fact that the concentrations of phosphate and of other constituents of the plasma vary. Intake of food increases the phosphate content of the plasma and the lymph, and so do numerous biochemical processes leading to an enzymic splitting of organic phosphorus compounds. The plasma phosphate, for example, increases after intense muscular action, though the low phosphate permeability of the muscle cells (Hevesy and Rebbe, 1940) much reduces the exodus of the phosphate ions split off during muscular action. On the other hand, excretion of phosphate acts in the opposite direction, and so do all those numerous biochemical processes in the course of which phosphate becomes incorporated with organic compounds, from which processes the decrease of the phosphate content of the plasma under the action of insulin is possibly the most conspicuous one (cf. Kjerulf-Jensen and Lundsgaard, 1943).

Not only do the phosphate and the equally important calcium concentrations of the plasma fluctuate, but the same applies to the concentration of phosphatase and other enzymes regulating the phosphorus metabolism. Such enzymes act on the bone formation not only by

regulating the phosphate concentration of the plasma, but possibly also in a more direct way, as suggested by ROBISON's (1912) early studies on bone formation. More recent work by ROCHE and MOURGUE (1939) leads to the result that a fracture of the rat's femur involves a loss of appreciable amounts of the mineral constituents of the femur followed by an opposite process after the lapse of about one month. In the first weeks, phosphatase activity of the bone is also enhanced. ROCHE and MOURGUE made the very interesting observation that the fracture of the left femur leads not only to an initial decrease in the mineral content of the fractured left bone, followed later by a reversal of this process, but a similar behaviour is also shown by the intact right femur. The enchanced phosphatase activity of the bone tissue may be due to an increased magnesium concentration produced by osteolysis following the fracture. Thus, even a fluctuation in the magnesium content of the plasma may promote the rate of renewal of the skeleton.

Fluctuations in the phosphate, calcium, magnesium, and phosphatase contents of the plasma thus make possible a biological recrystallization of bone apatite and, corespondingly, a renewal of the skeleton. That this process, which can be followed by making use of isotopic indicators, was found to be a slow one is easy to understand. The bone apatite contains a very appreciable part of the body's calcium and phosphorus contents, and these constituents are present in a crystalline state. Dissolution and formation of such crystallites may be expected to be a slow process. Furthermore, we must envisage the probability that a partial dissolution of a crystallite may be followed by a new formation of some molecular layers which protect the underlying part of the crystal from further changes. This process can often be repeated and leads to a repeated renewal of a fraction of the crystallites, while the remaining part of the crystal remains unchanged. The pronounced difference in the rate of renewal of epiphysial and diaphysial tissues found by various workers is due mainly to the better circulation taking place in the soft epiphysial bones but, possibly, to some extent to the smaller size of the crystals of the epiphysial tissue which favours an interchange between lymph (plasma) and bone phosphate.

In this paper are communicated the results of experiments carried out with the aim of measuring the rate of renewal of the mineral constituents of the fish skeleton. However, a short survey of the results hitherto obtained for the rate of renewal of the skeleton of mammalia will first be given.

RATE OF RENEWAL OF THE SKELETON OF MAMMALIA

As a result of the administration of labelled phosphate (phosphate containing a minute percentage of the radioactive phosphorus isotope ^{32}P), the "free" phosphate of the blood plasma soon becomes labelled, and the same applies to the extracellular fluid of the organism in view of the swift passage of phosphate ions through the wall's capillaries. As the plasma and the lymph contain labelled phosphate, all bone apatite formed after the administration of labelled phosphate is bound to be labelled. In the extreme case, when all mineral bone tissue is formed after the administration of labelled phosphate, 1 mgm bone P will have the same ^{32}P content, and thus the same radioactivity, as 1 mgm plasma P. Thus, the ratio of the specific activity (activity of 1 mgm) of the skeleton P and the specific activity of the plasma P is a measure of the rate of renewal of the skeleton.

When determining the rate of renewal we must take due regard to the fact that the specific activity of the plasma phosphorus does not remain constant, but decreases strongly in the course of the experiment, owing to successive interchanges of the plasma phosphorus with the phosphorus atoms of the various compounds present in the organs and also to excretion of phosphate. As the rate of interchange is different for different compounds and also for different organs, the calculation of the decrease of the specific activity of the plasma P with time encounters difficulties. The most direct way to eliminate the above mentioned difficulty is to administer repeatedly an appropriate amount of labelled phosphate, and, with the aid of this procedure, to keep the plasma phosphate at a constant level throughout the experiment. The results of such experiments (Hevesy *et alia* 1940) carried out on rabbits are seen in Table 1.

As seen from the figures, the degree of renewal of the mineral constituents of the skeleton in the course of 50 days amounts to 30 per cent

Table 1. Extent of Reneval of the Skeleton of a Rabbit in the Course of 50 Days

Fraction	Per cent renewed
Femur epiphysis inorganic P	29.7
Femur diaphysis inorganic P	6.7
Tibia epiphysis inorganic P	28.6
Tibia diaphysis inorganic P	7.6
Costa inorganic P	27.5
Scapula inorganic P	43.8
Femur epiphysis phosphatide P	100

in the case of the epiphysis of the tibia and to as little as 7 per cent for the diaphysis, while phosphatides extracted from the bone tissue are entirely, and possibly even several times, renewed in the course of the experiment.

The experiments mentioned above were carried out with fully grown rabbits, as in a growing organism the presence of labelled atoms cannot be interpreted exclusively as the result of a renewal process. It will also be due to the formation of *additional* tissue during the experiment. All molecules formed in a growing, labelled organism are, indeed, bound to become labelled. It is of importance, therefore, to carry out experiments on the renewal of the skeleton in adult animals.

The incorporation of labelled phosphate into mineral components of the bone is a very intricate process. Between the uppermost molecular layer of the apatite crystallites in contact with plasma or lymph, an exchange equilibrium can be established almost immediately. This means that the specific activity of the P present in these layers will promptly follow all changes in the specific activity of the plasma phosphorus. In most experiments with labelled phosphate, the active preparation is administered at the start of the experiment. After subcutaneous injection or administration by mouth, an increase in the plasma activity will take place in the initial phases of the experiment and a decrease throughout the later phases. Thus, the activity of the uppermost layer of the bone apatite is strongest in the early phases of the experiment in which the plasma is strongly active. Crystallites, however, formed in the course of the experiment from an active plasma, will contain comparatively large amounts of ^{32}P in view of the high phosphorus content of the total crystallites compared with the phosphorus content of the uppermost molecular layer. In view of the stability of the crystallites, much of their ^{32}P content will be conserved and will not follow or follow only slowly the changes in the activity of the plasma phosphorus.

Beside formation of entire crystallites from the labelled plasma we have also to consider the case of partial formation of crystallites. Some molecular layers are dissolved and replaced by layers formed by cristallization from labelled plasma. The newly formed layers will be active, but not the layers lying below. These layers will be protected from all action of the labelled plasma and, thus, from renewal. They will form a stable core for the crystallites and so will all crystallites that are not in contact with plasma or lymph.

Manly and his colleagues (1940), who carried out extensive studies into the uptake of ^{32}P by the mineral constituents of the bone where the activity was administered at the start of the experiment, estimate the share of labile and stable fractions of the bone tissue by comparing the activity of the blood (not of plasma) P and of the bone mineral of rats. They estimate $^1/_5$ of the ^{32}P content to be present after the lapse of 20

days in the labile portions of the epiphysis, the rest being found in the stable portion. The estimation of such magnitudes encounters great difficulties in view of the very complicated way in which the labelling of the bone tissue takes place. The degree of renewal of the mineral constituents of the different parts of the skeleton which takes place within a time interval can, however, be determined in the way described on p. 204. The degree of renewal in these experiments means the percentage of bone tissue newly formed once or several times since the start of the experiment.

RATE OF RENEWAL OF THE FISH SKELETON

We investigated the rate of renewal of the skeleton of sticklebacks *(Gasterosteus aculeatus)*. These fish, weighing 1—3 gm have a lifetime of about one year and can be expected not to grow any longer when one year old. Their small size has the advantage that the use of a large sea-water volume and, thus, an unduly large amounts of labelled phosphate, can be avoided. Our experiments, in which the sticklebacks were kept for up to six weeks in labelled sea-water, were carried out with radio-phosphorus having an initial activity of 0.05 millicurie, which was found to be ample to give an active skeleton.

The small size of the fish facilitates, furthermore, their ashing, which is to be carried out when we want to determine the total phosphorus content or the total ^{32}P content of the fish. Wet ashing was carried out by heating with 1 ml of conc. sulphuric acid containing some nitric acid and, in the last phase of the experiment, some hydrogen peroxide. 24 sticklebacks were kept in 3 litres of sea-water to which 10 ml of a radioactive solution containing 0.05 millicurie and 3.4 mgm of sodium phosphate (pH — 7.6) were added. After the lapse of 6 weeks, this activity declined to $^1/_8$ of its initial value. The water was daily renewed, as was its labelled phosphate content.

The sticklebacks were investigated at different intervals. After killing the fish and washing it very carefully with sea-water, the liver was taken, and the "free" phosphate was extracted from it with cold 5 per cent trichloracetic acid. The solution was then brought up to 25 ml. While the "free" phosphate content of 20 ml was precipitated as magnesium ammonium phosphate, the activity of which was measured, the residual part used in a colorimetric determination of the free P content of the extract. The activity measurements are much simplified when the samples have about the same weight. To obtain such samples, we added to the above mentioned 20 ml so much sodium phosphate that the precipitate obtained weighed 60 mgm. As mentioned above, the determination of the rate of renewal is based upon the comparison of the activity of 1 mgm of free plasma P and 1 mgm of mineral skeleton P. It is, however, extremely difficult to secure blood from fish weighing a couple of grams and, therefore, we replaced the determination of the activity of the plasma P by a determination of the activity of the free liver P. In view of the great ease with which phosphate ions penetrate the liver cells and vice versa, the activity level of the free phosphate P of the liver differs not much from the activity level of the free phosphate of the plasma. The writer is much indebted to Mr. Tryggve Gustavson for his very effective help in removing the livers, weighing 45—70 mgm.

To remove the organic constituents of the skeleton, we treated the bones with boiling glycol containing 6 gm KOH per 100 ml for several hours, until the bones

showed the total absence of non-mineral constituents. The bones were dried at 105° and dissolved in 2 ml 0.5 N HCl. The solution was brought to 25 ml and an aliquot was used, as described above, in the radioactive measurements, while the other aliquot was taken for colorimetric determination.

UPTAKE OF LABELLED PHOSPHATE BY THE FISH

The uptake of labelled phosphate by the fish most probably takes place either through the gills or through the digestive tract. While water passes the surface of the gills, some phosphate may reach the circulation. An increase of the water volume passing the gills may in this case be expected to lead to an increase in the phosphate uptake. Therefore, we have compared the phosphate uptake by fish kept in water rich in oxygen with the uptake of phosphate by fish in water containing but very small amounts of oxygen. While a group of fish was kept for a day in 2 litres of labelled sea-water saturated throughout the experiment, another group was kept in 2 litres of labelled sea-water to which no oxygen was added and in which other sticklebacks were previously kept in order to remove much of its air content. As seen in Table 2, the average uptake of ^{32}P by the two groups of fish practically does not differ.

TABLE 2. — UPTAKE OF ^{32}P BY FISH KEPT IN OXYGEN-RICH AND OXYGEN-POOR WATER IN THE COURSE OF 24 HOURS

Weight of fish in gm	Total activity present in 1 gm fish	
	water rich in oxygen	water poor in oxygen
0.82	20	
2.07	15	
1.77	25	
1.53	21	
1.25	17	
1.03	24	
1.15	16	
1.30	21	
0.91		23
0.88		21
1.28		13
2.63		19
1.71		26
1.51		22
1.24		20
1.56		24
Mean value ..	20	22

The fact that no significant difference was found in the uptakes of ^{32}P from waters rich and poor in oxygen does not prove conclusively that the main uptake of ^{32}P does not take place through the gills, as it is possible that the organism reacts to oxygen shortage in the water not by an increase in the water circulation through the gills, but by an enhanced oxygen extraction from the water. However, the above result induces us to draw our attention to the other probable way of entrance, the digestive tract, which, as marine fish drink large quantities of the water in which they swim, is the most probable path of ionic uptake by such fish.

Homer Smith (1930, 1931) added phenol red to aquarium water and found that the dye became concentrated in the intestine and also that it could not be absorbed through the gills and the skin. By measuring the concentration of the dye he was able to calculate the extent of water absorption taking place[1]. An eel weighing 143.5 gm, was found to have swallowed in the course of 20 hours 12.3 ml of sea-water. A number of experiments on eels and sculpins show that per kgm of weight those fish swallow from 40 to 225 ml of sea-water per day. The minimum swallowing observed is thus 0.04 ml per gm per day. The amount of labelled phosphorus present in 4.04 ml of water is that found by us in a stickleback weighing 1 gm after the lapse of 16 days. If the amount of water swallowed by the stickleback is not still larger than the largest amount observed in eels and sculpins, then we have to conclude that only a part of phosphate present in the swallowed water is absorbed by the sticklebacks. This result is by no means improbable. Though water is very easily absorbed, Homer Smith found that only 81 per cent of the water swallowed by the eel had been absorbed and, furthermore, that, while monovalent ions such as Na^+, K^+, and Cl^- were absorbed to a very large extent, divalent ions such as Ca^{++}, Mg^{++}, and $SO_4^=$ were not, these ions being concentrated in the rectal fluid. In relation to chlorine, sulphate was, for example, concentrated 24 times in the rectal fluid. (cf. A. Krogh, 1939). The result obtained by us suggests a similar fate of the phosphate ions.

A fish weighing 0.92 gm. took up in the course of 8 days $4 \cdot 10^{-3}$ per cent of the activity of the labelled sea-water and, thus, as the phosphorus content of the sea-water was 880 γ (730 added +150 present beforehand), 3.5 γ of phosphorus were taken up mainly as sodium phosphate by the fish. The amount of phosphate taken up by the fish during the same time varies appreciably from fish to fish. However, these variations do not influence the results obtained for the rate of renewal since, when calculating this magnitude, the skeleton activity and the liver activity of the same fish are considered.

[1] A detailed account of the work of Homer Smith is given by Krogh (1939)

Gasterosteus aculeatus is a fish provided with a skin armour[1]. In several cases, we investigated the armour, the cranium, and the vertebra separately.

EXTENT OF RENEWAL OF THE SKELETON

The method of calculating the extent of renewal of the skeleton is shown by the following example.

Duration of the experiment = 16 days. Average temperature = 16.6°. Fresh weight of the fish = 1.49 gm. Weight of the fresh liver = 50 mgm.

Free P content of the liver = 21 γ.

Total P content of the skeleton = 3.98 mgm.

Activity of the skeleton P = 29.2 counts per minute.

Activity of the free liver P = 8.8 counts per minute.

$$\frac{\text{Activity of 1 } \gamma \text{ skeleton P}}{\text{Activity of 1 } \gamma \text{ free liver P}} = \frac{0.00733}{0.418}.$$

Activity of 1 γ skeleton P in percentage of the activity of 1 γ liver P 1.8.

The figure obtained is not strictly identical with the percentage of the skeleton which, in the course of the experiment, is renewed, as we compared the specific activity of the bone P at the end of the experiment with the specific activity of the free liver P at the same date, while we should have considered the average value of the specific activity of the free liver P prevailing throughout the experiment. This magnitude is not known, but cannot be less than ½ of the final value. Therefore, we have to multiply, the result of 1.8 per cent arrived at by a figure which is less and probably appreciably less, than 2 in order to arrive at a correct renewal percentage which, thus, amounts to 3—4 per cent in the course of 16 days. This percentage of the skeleton was renewed once or several times, while the remaining 96—97 per cent of the skeleton remained unchanged.

The fish was kept in 3 litres of sea-water to which 3.4 mgm of labelled phosphate, corresponding to 0.73 mgm P and having an activity of $1.6 \cdot 10^6$ counts, were added (measured the same day as the activity of the liver and that of the skeleton). Water and activity were daily renewed.

1 γ free liver P was found to contain $\frac{1}{4 \cdot 10^6}$ part of the activity and, thus, from the free P extracted from the liver $2 \cdot 10^{-4}$ γ were such which originate from the labelled phosphate added to the sea-water. The total free liver P contained $\frac{1}{1.8 \cdot 10^5}$ part of the activity added to

[1] A detailed description of the armour is given by F. Roth (1920).

the water. The skeleton contained $1/5.3 \cdot 10^4$ part of the activity added to the water, thus of the 3.98 mgm P present in the skeleton, $1.4 \cdot 10^{-2}\gamma$ were such which originate from the labelled phosphate added to the sea-water.

The results obtained for different parts of the skeleton are computed in Table 3.

Table 3. — Percentage Ratio of the Activity of 1 mgm Skeleton P and 1 mgm Free Liver P

No. of experiment	Fresh weight of the fish in gm	Time in days	Part of the skeleton	Percentage ratio of the activity of 1 mgm skeleton P and 1 mgm liver P
			Skull	1.7
19	1.83	17	Vertebrae	1.1
			Armour	1.2
18	2.20	20	Armour	2.3
21	1.78	20	Skull	2.7
			Armour	2.1
			Skull	2.4
22	1.21	21	Vertebrae	3.4
			Armour	2.6
23	1.33	30	Vertebrae	4.1
			Armour	2.4
24	1.41	31	Armour	4.2

From the above figures, the average value for the percentage ratio of the activity of 1 mgm skeleton P and 1 mgm liver P works out to be 2.5 per cent in the course of 22 days. To obtain the percentage renewal of the fish skeleton in the course of 22 days, we have to multiply the above figure (cf. p. 209) by a figure which is less than 2. The rate of renewal thus lies between 2.5 and 5 per cent.

UPTAKE OF LABELLED PHOSPHORUS BY THE EGGS

In several fish, a large number of eggs was found and in many cases the weight of the eggs constituted a very appreciable percentage of the weight of the fish. In experiment No. 16 B, the eggs' weight was found to be 0.92 gm out of a weight of 2.44 gm of the fish (including eggs), i. e. 38 per cent. The percentage ratio of the activity of 1 mgm average fish P (minus eggs) and 1 mgm free liver P was found to be 6.3. For the percentage ratio of the activity of 1 mgm egg P and 1 mgm liver P, 90 was obtained. Thus, almost all P of the atoms present in the egg was incorporated into the eggs in the course of the last 23 days. The total P content of the

fish without its eggs was found to be 17.78 mgm, or 1.17 per cent, while the corresponding figures for the eggs were 2.7 and 0.29.

Fish No. 16 had a weight of 1.07 gm including its eggs, which made up 40 per cent of the total weight. Liver and heart were strongly degenerated, the liver weighing only a few milligrams. The duration of the experiment was 16 days, the activity of the eggs amounting to 12.8 per cent of the total activity. In the above case (No. 16 B), the corresponding ratio was 18.3. Thus, after the lapse of 16 days, only about $^2/_3$ of the egg P was found to be incorporated in the eggs in the course of the experiment, while in experiment No. 16 B., after the lapse of 23 days, almost the whole P content of the eggs was deposited (through growth or renewal) in the course of the experiment.

In experiment No. 16 A., the percentage of P taken up in the course of the experiment from the water was found to be $1/3.9 \cdot 10^4$ of the amount present, corresponding to $2.2 \cdot 10^{-2}$ γ.

In experiment No. 16 B, $1/2.2 \cdot 10^4$ of the water P was taken up by the fish amounting to $4.4 \cdot 10^{-2}$ γ.

DISCUSSION

The average value for the degree of renewal of the fish skeleton in the course of 22 days was found to be 2.5—5 per cent, the lower value being the more probable. This means that, while 2.5—5 per cent of the skeleton were renewed once or several times — a part of this percentage was presumably renewed frequently — at least 95 per cent of the skeleton remained entirely unchanged. When arriving at this conclusion, we assumed that no additional growth of the skeleton took place in the course of the experiment. As such additional growth would take place from a labelled plasma, all newly formed skeleton might be expected to be labelled, and what we interpreted as a renewal of the skeleton might in such a case be due to additional bone formation in the course of the experiment. We could not find any evidence for a growth of the skeleton or a growth of the fish taking place in the course of the experiment. It is very difficult, however, to exclude the possibility of an increase in the mineral constituents of the skeleton by a few percent. The above mentioned 95 per cent therefore have to be considered a lower limit for the part of the skeleton remaining unchanged after three weeks.

In the case of the fully grown rabbit, about 10 per cent of the skeleton were found to be renewed in the course of only 9 days (Hevesy *et al.* 1940). The great difference in the renewal rate of the skeleton of the rabbit and that of the fish is presumably due to the great difference in the body temperature. The renewal of the skeleton is partly a "physical" replacement process between the phosphate of the uppermost

molecular layer of the bone crystallites and the phosphate of the plasma, and partly the effect of a "biological recrystallization". Crystallites or parts of crystallites go into solution and new crystallites are wholly or partly formed by crystallization from the plasma. In experiments of long duration, the interchange mostly takes place by biological recrystallization. Now, such a process may be expected to be strongly influenced by the body temperature and to take place at a higher rate at 37° than at 16°.

It is interesting to note that in experiments of only a few hours' duration, increase of temperature was found to promote the radio-phosphorus uptake by the bones. The tibia of the frog (Hevesy *et al.* 1940) was found to take up nearly 1½ times as much radio-phosphorus at 22° as at 0°.

We found that a fish weighing about 1 gm took up, in the course of 16 days, $1/4 \cdot 10^4$ part the phosphorus added to the water i. e. $2 \cdot 10^{-5}$ mgm. As the water volume was 3000 ml., the amount of P taken up by the fish from water is equivalent to the amount of P present in 1/13 ml water. The amount of water taken up by the fish in the course of 16 days is presumably much larger than 1/13 of its body weight (cf. p. 206) and, thus, only a minor part of the P content of the water swallowed is absorbed.

Summary

Sticklebacks *(Gasterosteus aculeatus)* were kept for periods of up to one month in 3 litres of sea-water containing labelled phosphate. A fish weighing 1 gm was found to take up in the course of 16 days $\frac{1}{40.000}$ part of the phosphorus present in the water, corresponding to $2 \cdot 10^{-5}$ mgm P.

By comparing the specific activity of the skeleton P with that of the free liver P, figures for the degree of the renewal of the skeleton were obtained. At least 95 per cent of the skeleton were found to remain unchanged during the experiment. The rate of renewal of the fish skeleton is thus much lower than that of the mammalian skeleton.

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Comment on papers 18—23

The first application of an artificial radioactive isotope as a tracer in life sciences aimed to determine if and to what extent the mineral constituents of the skeleton of the fully grown rat are renewed. ^{32}P was used in this investigation (paper 18), which demonstrated the renewal of an appreciable part of the mineral constituents of the skeleton of the rat. The replaceable fraction of the bone phosphate was found to constitute about 30 per cent, a result which was confirmed by later investigations. This study was carried out and its result published simultaneously (1935) with Schoenheimer and Rittenberg's first classical investigation on the dynamic nature of fat deposits, followed by numerous others of a similar type. The result that the formation of the bone is a dynamic process, involving continuous loss and replacement was for many an unexpected and puzzling one. This is shown by a remark of the Editor of *Nature*. In those days, each issue of *Nature* contained a short survey of the contents of the "Letters to the Editor". In this survey it is stated: ". . . . The authors (Chiewitz and Hevesy) further believe (!) that the formation of the bone is a dynamic process involving continuous loss and replacement." A larger fraction of the epiphysial apatite molecules than of the diaphysial ones was found to be renewed, which is partly due to the fact that mineral molecules of the epiphysial tissue come more easily in contact with plasma and lymph containing the active phosphate than those of the more perfectly mineralized diaphysial tissue. The first animals investigated included also rachitic rats (paper 19). At first the puzzling result that the fraction of bone phosphate renewed in the rachitic rat was found to be larger than in the healthy animal, was at least partly due to the less well mineralized and thus more epiphysial nature of the rachitic skeleton. The uptake of minute amounts of ^{32}P by the enamel was found to take place even when preventing contact between enamel and saliva (paper 20).

Since the specific activity of plasma phosphate diminishes with time, the sensitivity of the radioactive indicator correspondingly decreases (the same activity indicates a larger amount of phosphorus). To arrive at an exact figure for the renewed fraction of the apatite phosphate, we must know the specific activity of the plasma inorganic phosphate throughout the experiment. An alternative method is to keep the plasma inorganic phosphate activity at a constant level throughout the experiment. The ratio $\frac{\text{apatite } ^{32}P \text{ spec. activity}}{\text{plasma inorganic } ^{32}P} \times 100$ determined at the end of the experiment indicates then the percentage apatite renewal. By following the last mentioned procedure (paper 21), after the lapse of 56 days 30 per cent of the femur epiphysis phosphorus, but only 7 per cent of the diaphysis phosphorus of the rabbit, was found to be renewed. A 44 per cent renewal of the scapula phosphate took place, and a full renewal of the apical and medial incisor phosphate occurred. At least two-thirds of the last mentioned renewals was due to incisor growth. The constancy of the serum-calcium and serum-phosphorus content of the plasma is maintained by homeostatic mechanism, for which the skeleton is primarily responsible. The regulation is achieved mostly by a slight change in the amount of dissolved and newly formed bone apatite.

While in the above mentioned investigations the dynamic nature of a substantial part of the skeleton apatite was demonstrated and the fraction of the latter

which participates in a renewal process determined, information on more detailed processes taking place in the skeleton were available only after the introduction of the autographic methods of investigation in this type of study by LEBLOND *et al.* (1951).

The ^{32}P applied in our early investigations was prepared under the action of neutrons emitted by radon-beryllium sources on 10 l. of CS_2, the ^{32}P being extracted by strongly diluted acid. On his fiftieth birthday, Professor Niels BOHR was presented by his friends with 600 mgm of radium, which he most generously put at our disposal. It was the Union Haute Minière which supplied the radium as sulphate mixed with 2 gm of beryllium. In view of the hygroscopicity of $RaCl_2$ the Union Haute Minière was at that early date not willing to supply $RaCl_2$-beryllium mixtures. After the availability of cyclotron-produced ^{32}P, Professor Ernest LAWRENCE most kindly repeatedly mailed to us, starting in 1937, a few millicuries of ^{32}P prepared by Dr. Martin KAMEN. This was a very great help, as was ^{32}P supplied later by Professor BOHR's and Professor SIEGBAHN's cyclotron.

In paper 19, published in 1937, the first clinical investigation is described in which a radioactive isotopic indicator was applied, the determination to what extent the phosphorus of the faeces is of endogenous origin and the investigation of the incorporation of ^{32}P into the placenta. Paper 20, published in the same year, contains data on the incorporation of ^{32}P into human teeth, both into roots and crowns. Deuterium, thus a stable isotopic indicator was formerly used (paper 54) in the determination of the water content of the human body. In 1937 HAMILTON published a study on the rate of absorption of sodium by fasting human subjects following the oral administration of labelled sodium. BORSOOK *et al.* described in the same year the excretion by human subjects of administered ^{35}S.

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24. CONSERVATION OF SKELETAL CALCIUM ATOMS THROUGH LIFE

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Dedicated to Professor Niels Bohr on the occassion of his 70th birthday

From the earliest beginning, Professor Niels Bohr has shown great interest in the application of radioactive indicators to the study of the conservation of skeletal atoms through life. This fact has induced the writer to contribute to this volume with a communication of the results obtained in an investigation on the conservation of skeletal calcium atoms in the adult mouse and on the fate of maternal calcium atoms through generations.

The first application of an artificially radioactive isotope as a tracer in 1934 was that of ^{32}P in a study of the problem whether and to what extent the mineral constituents of the skeleton of the adult organism are replaced during lifetime[1, 2, 3, a]. By using this radioactive indicator it was possible to demonstrate the dynamic nature of the building up of bone tissue. It was found that an initial rapid location of the circulating labelled phosphate in the mineral constituents of the skeleton is followed by a slower second effect. The first effect was interpreted by us to be due to an interchange between the phosphate ions located in the surface layer of the bone apatite and in the plasma, the second one, however, to the fact that "the bone is destroyed at certain places and rebuilt under incorporation of labelled phosphate at others". Emphasis was given to the analogy between these phenomena and those observed when, in early experiments, naturally radioactive isotopes were applied as tracers. Paneth[4], when shaking solid sulfate with a solution containing labelled lead ions, observed an interchange of lead ions only between the uppermost molecular layer of the solid salt and the dissolved ions. In studies in which the interchange between the atoms of lead metal and the labelled lead ions of a solution, or vice versa, was investigated, the present author and others[5, 6, 7] found that many hundreds of atomic layers of the lead foil were converted into ions, and a corresponding number of ions into atoms, making out the lead foil. Thus, a renewal of the constituents of a metal foil, involving dissolution and reprecipitation due to "local currents", was found to be a much deeper going process than that occurring between solid lead salt and the

lead ions of the sorrounding solution. In the early investigations mentioned above, it was pointed out that the rapid uptake of ^{32}P during the early phase of the experiment recalls the behaviour of a lead salt placed in the solution containing labelled lead ions, the recrystallization of the mineral constituents of the skeleton reminds of the behaviour of a lead foil immerged into a solution containing labelled lead ions, however, with the difference that, in the latter case, enzymic actions are involved. Or, as it was expressed later[8], "A restricted extent of renewal of the skeleton is due to the fact that, while the P atoms of the uppermost molecular layer of the bone apatite crystals can promptly interchange with the free P atoms of the plasma (actually not the P atoms, but the phosphate ions interchange), a renewal of the main part of the apatite P can take place only when the crystal is dissolved and when new crystals are formed from the plasma; from labelled plasma, labelled crystals are formed". Subsequent experiments confirmed the correctness of these early conclusions, showing that both a surface exchange between plasma phosphate and bone phosphate, and a recrystallization, thus a dissolution of some of the apatite crystals and the formation of new ones, take place in the skeleton. Different workers, however, arrived at divergent results about the share of both processes in the interaction of plasma and bone constituents.

The introduction of autoradiographic methods into the study of bone formation by LEBLOND et al.[9]. was a very important advance, since it became possible to visualize the rapid formation and destruction of some parts of the calcified tissue. Numerous autoradiographic investigations such as those by LEBLOND et al. applying ^{32}P, those by COMAR et al.[10] using ^{45}Ca, ^{89}Sr, and ^{32}P, by SKIPPER et al.[11] with ^{14}C, by KIDMAN et al.[12] with ^{89}Sr, by ENGFELDT et al.[13] with ^{32}P, by AMPRINO and ENGSTRÖM[14] with ^{45}Ca, and by BAUER[15] with ^{22}Na, clearly demonstrate that a great part of the bone salt crystals are more or less unchanged until they are reached by the process of resorption.

LEBLOND's autoradiographs clearly indicate that the circulating phosphate enters the skeleton either by *exchange* or by *precipitation* in definite areas with the formation of new bone. While, in the autoradiographs, the exchangeable phosphate is depicted as diffuse reactions disappearing rapidly with time, the precipitated or stable phosphate appears as localized persistent reaction.

In contradistinction to all workers in this field, ENGFELDT, ENGSTRÖM and ZETTERSTRÖM[13] arrived at the result that even the initial uptake of ^{32}P by the bone is due exclusively to some kind of recrystallization. This conclusion is based on their observation that the autoradiographic patterns of cross sections from long bones show an uneven distribution of radioactive phosphate. They found the fastest uptake of labelled phosphate to take place in Haversian systems with a low content of mine-

ral salts. Since the major part of the tracer is found in limited areas, the initial rapid uptake of labelled phosphate — according to their view — cannot be due to ion exchange on the crystal surface of the bone minerals, such an exchange being prevented by the organic constituents of the bone.

While there can hardly be any doubt that the main part of the renewal of the bone apatite of skeleton is due to a recrystallization process, to a degradation and new formation of the mineral constituents of the skeleton, objections may be raised against the view that the initial uptake of ^{32}P is due exclusively to some kind of recrystallization.

Uneven distribution of radioactive phosphate as shown in autoradiographic patterns of cross sections from long bones cannot be interpreted as an absence of surface interchange. According to PANETH[4, 16], the whole uppermost molecular layer of crystalline salt powders interchanges with the ions of a surrounding solution, while properly crystallized surfaces like those of natural crystals fail to do so. He states that his investigations suggest that the radioactive method of determining surfaces, based on the assumption that the whole uppermost layer molecular interchanges, should be employed in those cases only for which it is established that the fundamental supposition of kinetic exchange of the entire surface is valid. If we assume the bone apatite, or part of it, as occurring *in vivo*, to be a properly crystallized substance, we arrive at another explanation than that of ENGFELDT, ENGSTRÖM and ZETTERSTRÖM, according to which the organic constituents of fresh bone are responsible for preventing surface exchange. This alternative explanation is that the bone apatite, or large parts of it, behaves like a properly crystallized substance in Paneth's experiments and not like a crystal powder.

The exchange of ions on a crystal surface is thus far from being absent and, though restricted to a fraction of that surface, is responsible for an appreciable part of the early uptake of labelled ions by the mineral constituents of the skeleton. As shown by ARMSTRONG and assoc.[17], in the course of the first ten minutes, 2% of the skeletal calcium of the dog are replaced by labelled calcium of the plasma; this is $^1/_{10}$ only of the amount which, according to FALKENHEIM's[18, 18a] calculations, would be necessary to replace the whole uppermost molecular layer of the bone apatite, or $^1/_6$ of the amount estimated by HENDRICKS and HILL[19]. A large part of these 2%—or even 2%—could be due to a surface interchange in spite of the uneven autoradiographic patterns of cross sections of long bones observed by ENGFELDT, ENGSTRÖM, and ZETTERSTRÖM. In view of the high specific activity of the plasma calcium in an early stage of the experiment, to a 2% interchange corresponds a very much higher percentage decrease in the ^{45}Ca content of the plasma in the course of the first two minutes, more than 50% of the injected radio-

calcium leaving the circulation. From Ca^{45} injected into the circulation of growing hogs, COMAR and assoc.[10] found only 2% to be present after the lapse of an hour.

In the experiments of ENGFELDT and assoc., three hours was the shortest time after which the ^{32}P injected rats were sacrificed. The early phase of the experiment, in which a very rapid interchange of the mineral constituents of the bone takes place, is much shorter than three hours. When investigating the uptake of ^{89}Sr by the skeleton of outgrown rabbits during the first 30 seconds, 11.7% were found to be taken up[20], while during the first six hours—thus a 720 times longer period—only about twice as large an uptake was observed. ARMSTRONG and assoc.[17] found during the first 20 minutes an interchanges of 4% of the skeletal calcium with plasma calcium, this amount increasing less than three times during the following 160 minutes.

A change in the concentration of the bone apatite constituting ions, and still more a variation in the concentration of enzymes involved in recristallization of the plasma and the lymph, is bound to influence the rate of recrystallization of the skeleton. Repeated administration of bone phosphate extract by intravenous injection was found to lead to a decrease of the mineral constituents of the bone tissue[21], which are replenished after removal of the excessive phosphatase. HASTINGS[22], when replacing the plasma of a dog by plasma of low calcium content, found that the mobilization of bone calcium increased the calcium level of the plasma almost momentarily to a normal level. Parathyroid hormon is known to exert a direct action on bone[22a]. In this connection, also CARLSON'S[23] investigation should be mentioned; he found vitamin D deficient rats to be unable to utilize their bone stores for maintaining a normal serum calcium. However, in view of the very great difference in the distribution of the mineral constituents in the bone tissue and the corresponding tendency to remove these differences, the biological recrystallization of the skeleton, as rightly emphasized by ENGFELDT and assoc.[13], is not due exclusively to these processes[24].

KING raised the idea that, though conventionally, the bony framework of the body is regarded as a means of making locomotion possible, it may be that this is no more than a secondary development, the primary function of bone in the body being to act as a reservoir for the maintenance of a constant blood calcium level.

At a very early date[25, 26, 3], it has already been observed that the diaphysial phosphate is replaced by administered labelled phosphate at an appreciably lower rate than epiphysial phosphates, and similar observations were made in the investigation of the incorporation of ^{45}Ca into the skeleton[27, 29]. Since the transition between diaphysial and epiphysial bone tissue is almost continuous, the specific activity of bone phosphorus or bone calcium varies considerably through the whole

bone tissue. This great heterogeneity of the specific activity of the bone apatite phosphorus could be demonstrated by ZETTERSTRÖM and LJUNGGREN[30] by isolating bone fractions of different solubility and measuring their specific activity. The most soluble bone phosphorus was found to show the highest specific activity, thus the most rapid rate of renewal. X-ray absorption and diffraction studies by AMPRINO and ENGSTRÖM[11] revealed also that the distribution of mineral components in the bone tissue is far from being uniform.

SIZE OF THE NON-RENEWABLE PART OF THE SKELETON

The extent of renewal of apatite phosphate of the skeleton can be calculated from the mean value of the specific activity of the plasma phosphate during the experiment and the value of the specific activity of the apatite phosphate at the end of the experiment. During the early part of the experiment, the sensitivity of the radioactive indicator is comparatively low, thus a strong decline in the plasma activity corresponds to a comparatively low interchange figure. In the later part of the experiment, the same activity which indicated at the start the presence of 1 mgm of phosphorus in the plasma, for example, indicates 100 mgm, thus the sensitivity of the radioactive indicator is strongly increased. Now, a further interchange will be indicated by a very small further loss of activity. Furthermore, following the interchange of plasma and bone phosphate for a longer time interval, increase and decrease in the specific activity of the plasma phosphate may alternate due to a variation in the phosphate intake or other reasons. Thus, it encounters difficulties, by comparing the mean specific activity of the plasma phosphate during the experiment and the specific activity of the apatite phosphate at the end of the experiment, to find a reliable value for the extent of the renewable part of the mineral phosphate of the skeleton, and similar considerations apply to the determination of the renewable part of bone calcium in contrast to that of the bone sodium. Sodium, being mainly an extracellular element, is distributed between plasma and extracellular fluid within a few minutes, a distribution which results in a decrease in the specific activity of plasma sodium to about $^1/_6$ of its original value, followed by a slow decrease with time only. Thus, as discussed on p. 16, the extent of the renewable part of the mineral bone sodium could be calculated from specific activity data. We can, however, determine the extent of renewal of bone phosphate from specific activity data when keeping the specific activity of the plasma phosphate at a constant level during the experiment. This result was obtained by the author and his associates[31] by daily

injecting the rabbit repeatedly with labelled phosphate. After the laps of 50 days, the phosphorus of the femur epiphysis found to have a specific activity of 30% of that of the plasma inorganic P, thus indicating that 30%, and only 30%, of the epiphysial bone apatite had been renewed, a much lower renewal figure (7%) being obtained for the diaphysial phosphorus.

This method has the disadvantage of being cumbersome. Furthermore, the results may be influenced by the time that passes between the last injection of the rabbit and the killing of the animal. Therefore, when determining the renewable fraction of the skeleton calcium of the mouse, we have chosen another procedure. Mice were bred whose skeleton was labelled throughout with ^{45}Ca and the loss of the activity in the skeleton was followed with increasing age of the animals. Such mice can be obtained by administering to the mother food containing labelled calcium already weeks before gestation and continuing to feed the lactating mother and the growing offspring with food containing labelled calcium. We assume every one of the offspring to have the same ^{45}Ca content. If we stop administering labelled food after these offspring are outgrown, they start to interchange their labelled bone calcium with the unlabelled calcium from the food with the result that the ^{45}Ca content of the skeleton decreases and, when the offspring is killed after two months, its ^{45}Ca content is lower than that of another offspring killed after one month. By killing members of a litter at different dates, we can follow the processes in the skeleton for years, viz. through the lifetime of the animal.

Table 1. — Weight and Activity of New-born Mice

No.	Weight in gm	Relative activity
1	1.8	100
2	1.3	98.5
3	1.4	93.5
4	1.2	99.5

The ^{45}Ca content of every member of a litter is not strictly the same, and this applies also to the growth rate. The evidencee that a part of the curve depicted in Fig. 1 (p. 224) is discontinuous may presumably be due to a difference in the uptake of maternal ^{45}Ca by the offspring of the same litter. The variation in the radioactivity of different members of a litter, however, is restricted and does not suffice to frustrate the applicability of the method described (cf. Table 1).

EXPERIMENTAL

In view of the difficulties in replacing all food calcium by labelled calcium, we added the labelled calcium as $CaCl_2$ (150 mgm. per liter) to the drinking water, on the assumption that the quantity of water drunk by the mouse, kept at constant temperature, is about proportional to the intake of food which consisted of standard cakes. We started to administer two to ten weeks before parturition to 20—30 gm mice the labelled $CaCl_2$ and continued administration of such drinking water till weaning. Then, the growing mice were given labelled drinking water until they were outgrown. From that date (when the mice were about 100 days old), administration of ^{45}Ca was discontinued. The offspring were killed at different times, and the radioactivity of the ash of their skeletons was compared. 20 mg. of bone ash were placed under the Geiger counter, and the total activity of the skeleton was calculated from the measured activity and the total ash weight. In other experiments, the radioactivity of the total body ash samples was compared.

The ratio of the activity of 20 mgm of bone ash of outgrown and of newborn mice is not a correct measure of their relative ^{45}Ca content. The calcium content of the ash of the newborn being appreciably lower than that of the adult, the backscattering of the β-rays emitted by the ^{45}Ca of the first mentioned samples will be lower, furthermore the consistency of the samples and, thus, the distance of the sample from the counter window may slightly differ. By measuring once the activity of a 20 mgm sample of the bone ash of newborn mice, and then that of a small known aliquot of this sample brought up to 20 mgm through addition of inactive bone ash of an adult mouse, we arrive at the result that the activity measured of the ash of the newborn mouse has to be multiplied by 1.05 in order to make it comparable with the activity of the bone ash of adult mice.

In other experiments, new-born mice were shifted from their active mothers to inactive mothers shortly after birth; determinations were made of the percentage of maternal labelled calcium taken up by the offspring after birth and the rate of loss of these calcium atoms during growth and later.

The ^{45}Ca activity of the mice remained below 0.05 μC per gm and, in most cases, it was very appreciably less. SIMMONS and assoc.[32] observed the effects of radiation produced in mice during 108 weeks. When a dose of 0.034 μC per gm was administered, they could not find anaemia; when the dose was raised to 0.068 μC.

TABLE 2. — COMPOSITION OF CAKES FED TO OUR MICE ("GARD-BRED")*

100 gm cakes contain	
Water	7.6 gm
Ash	2.9 ,,
Proteins (5.7 × N)	6.3 ,,
Carbohydrates	67.6 ,,
Ca	188 mgm
P	424 ,,
Fe	24 ,,
Combustion value (calculated according to Rubner)	400 cal.

* In Sweden, mice and rats are fed almost exclusively on these cakes, the exact composition of which was hitherto unknown. The author is much indebted to Professor E. BRUNIUS and Mrs. ESTHER SIHLBOM who most kindly made the analysis of these cakes at Statens Institut for Folkhälsan.

moderate changes in heterophylous values could be detected. In our experiments, no effect on growth or fertility due to the presence of ^{45}Ca could be observed. Our main litter size was 5.7. As shown by RUSSELL[33], the litter size of mice at term is reduced as a result of irradiation during preimplantation stages with 100 r or more, and when exposed shortly after implantation, by a minimum dose of 200 r.

The composition of standard cakes fed to our mice is seen from Table 2.

RESULTS

The results of experiments in which mice born from mothers kept on a ^{45}Ca diet for weeks prior to and after parturition, and continuously kept on a ^{45}Ca diet till they reached an age of about 100 days, thus were fullygrown, are shown in Table 3.

The mean conservation of ^{45}Ca by the uniformly labelled skeleton of the mice in the course of 390 days, representing a mean value of the duration of the experiments, works out to be 64.7 ± 7.34 per cent, the standard error of the mean being 2.78. If we disregard the last experiment in which the mice were kept on a high calcium diet, the mean value is 67.2 ± 7.86 per cent, the standard error of the mean being 3.23. Thus, $^2/_3$ of the calcium atoms present in the skeleton of the out-

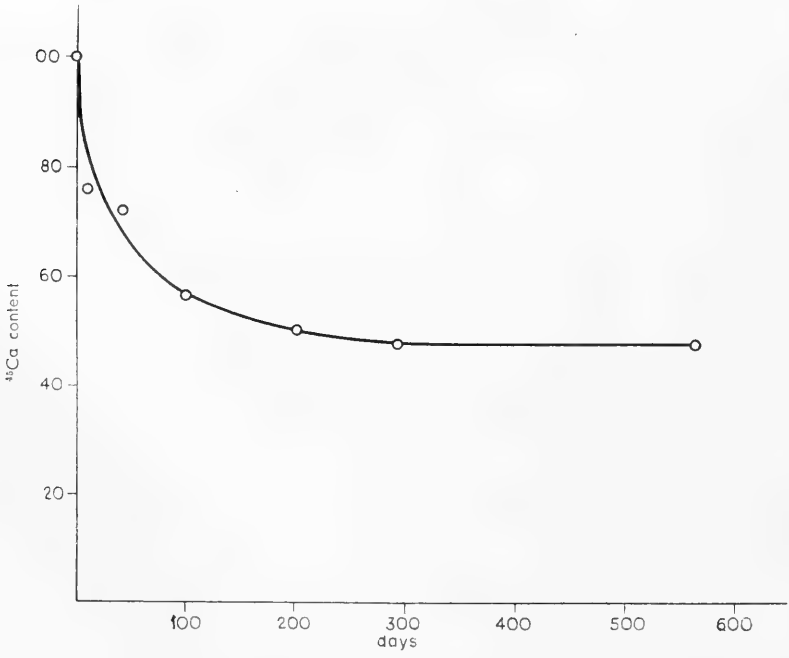

FIG. 1. Loss of ^{45}Ca, obtained from labelled mother at birth, during lifetime of the mice. Each point indicates the ^{45}Ca content of another member of the litter killed at the stated time

TABLE 3. — LOSS OF ^{45}Ca BY THE UNIFORMLY LABELLED SKELETON OF MICE WITH TIME INDICATED BY MEASUREMENTS OF THE RADIOACTIVITY OF THE SKELETON OF DIFFERENT MEMBERS OF A LITTER KILLED AT VARIOUS TIMES. THE MICE WERE BORN FROM ACTIVE MOTHERS AND WERE ADMINISTERED ^{45}Ca UNTIL THE FIRST MEMBER OF THE LITTER WAS KILLED

No. of litter	Age in days	^{45}Ca content
I	111	100
	329	66.7
	519	57.0
II	108	100
	327	90.7
	517	78.8
III	108	100
	326	88
	501	69.4
IV	115	100
	220	79.9
	393	64.4
V	106	
	106	100
	231	66
	325	63.8
VI	56	100
	129	81.4
	266	69.7
VII*	99	100
	214	77.7
	308	55.5
	392	55.9
	503	50.1

* Cheese and egg shells were added ad lib. to the standard "gard-bred" diet.

grown mice are present after the lapse of more than a year and can thus be considered to be unreplaceable during life.

Figs. 1 and 2 and Table 4 show the results of some of our experiments in which the litter, born from active mothers, was kept from birth on a ^{45}Ca-free diet. These experiments include the results obtained between the third and the 560th day after birth, thus almost the lifetime of the mouse. The percentage of ^{45}Ca lost between day 3 and day 560 works out to be 53 per cent and 44 per cent, respectively. The mean loss of

^{45}Ca observed in experiments lasting 100 to 180 days amounts to 43 per cent (Table 4). The loss of ^{45}Ca during the first three days of life is less than 10 per cent; thus half of the maternal calcium atoms are preserved during life.

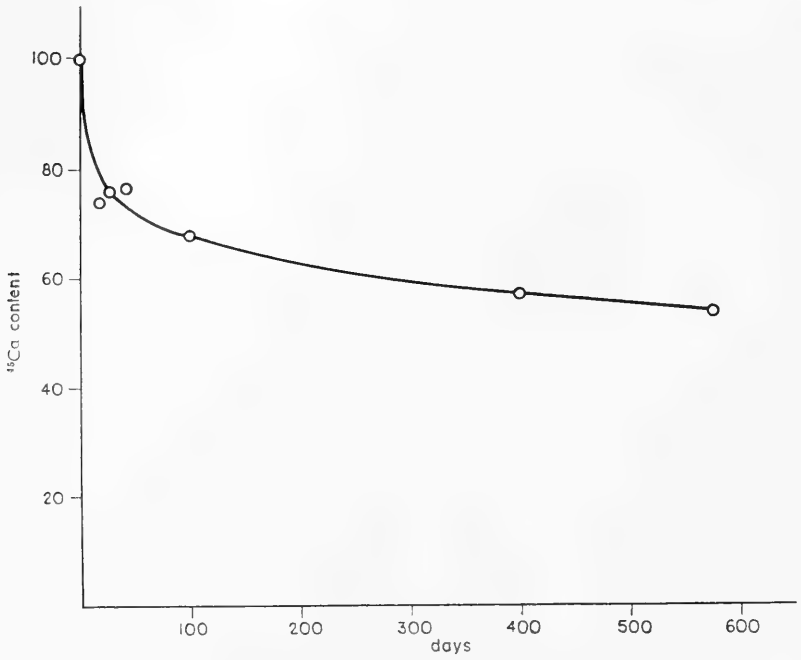

Fig. 2. Loss of ^{45}Ca, obtained from labelled mother at birth, during lifetime of the mice. Each point indicates the ^{45}Ca content of another member of the litter killed at the stated time

The calcium content of our newly born mice, weighing 1.23—1.37 gm. varied between 0.28 and 0.35 per cent of the body weight, not much differing from the calcium content of the new-born rat (4.7 gm) for which data varying between 0.27 and 0.35 per cent are reported[42]. The calcium content of 1 gm fresh weight of newly born mouse amounts to 0.3 times that of 1 gm of the adult animal, which is 1.05 per cent. If all the maternal calcium atoms had the same chances to supply calcium to the offspring, and all were labelled, we would find 1 gm of newly born mouse to be 0.3 times as active as 1 gm of the mother. We find the ^{45}Ca content of 1 gm of new-born mouse to amount to 1.7 times that of 1 gm of the adult mouse. The ^{45}Ca taken in by the mother has thus only an opportunity of interchanging in the average with about $^1/_5$ to $^1/_6$ of the body calcium before being utilized in the building up of the embryo.

TABLE 4. — RETENTION OF MATERNAL CALCIUM ATOMS BY THE OFFSPRINGS

No. of litter	Age in days	Weight in gm	Per cent of mothers' activity present in the offspring	Number of maternal atoms present
I	3	2.95	8.25	100
	31	13.9	6.59	80
	39	16.8	5.50	67
	43	18.6	6.0	72
	103	24.3	4.25	52
II	1	2.20	6.95	100
	129	41.9	4.95	71
	181	31	3.96*	57
III	1	1.80	10.3	100
	131	38.1	7.4	72
IV	1	1.45	9.91	100
	128	39.6	7.25	73
	181	40.0	5.69	57
V	3	3.1	8.55	100
	27	11.5	6.98	82
	35	21.0	6.67	78
	42	24.2	6.27	73

DISCUSSION

a) Conservation of the calcium atoms of the outgrown skeleton through life

The fact that a very appreciable part of the skeletal calcium is preserved in the outgrown animal throughout its lifetime results from experiments carried out by SINGER, ARMSTRONG and PREMER[34], by CARLSON[28,35] and by BAUER[15a]. Similar results were obtained in investigations on the renewal of the mineral constituents of the skeleton, performed by the present author and his assoc. who used ^{32}P as an indicator[31].

From specific activity data of the plasma and the skeleton of the outgrown rat, the percentage of renewable skeletal sodium was calculated by BAUER[36a] to amount to 30—40 per cent of the sodium present (disregarding the extracellular sodium); a similar figure — 45 per cent — is reported by EDELMAN[38] and by BADEN and MOORE[39]. Since sodium is mainly an extracellular element, the specific activity of plasma sodium decreases only slowly with time, not so the specific activity of calcium. The calculation of the percentage of renewable skeletal calcium from

specific activity data is therefore encumbered with difficulties (cf. p. 221). From data collected during five days, BAUER[15] estimates, however, that less skeletal calcium than skeletal excess sodium is exchangeable in the rat, thus less than 30—40 per cent. As it was shown above (p. 217), the mobilization of some further skeleton calcium is still going on in the mouse after the lapse of more than 100 days and the non-exchangeable part of the skeleton amounts to 67 per cent.

It is interesting to note that, when injecting ^{45}Ca at the start of the experiment interperitoneally to outgrown rats whose skeletal calcium content was increased appreciably during the experiment, SINGER and ARMSTRONG[40] found a ^{45}Ca retention of 42—45 per cent in the skeleton after the lapse of 52 days and the release of only small amounts of radiocalcium after that date. BUCHANAN[41], who exposed mice to air containing $^{14}CO_2$, found that 30 per cent of the bone carbonate are replaced within 12 days, while 45 per cent only are renewed in the course of three months.

b) Conservation of maternal calcium atoms by the offspring through life

Our results demonstrate the very pronounced ability of the skeleton to conserve maternal atoms.

In the first mentioned experiments, one third of the ^{45}Ca content of the outgrown mouse was found to be replaceable by inactive food calcium. In the latter experiments with growing mice, released ^{45}Ca had a further outlet, viz. utilization in the formation of additional skeleton, which takes place in the growing organism.

Investigations were carried out earlier on the loss of ^{32}P through the lifetime of mice born from active mothers[37]. Some results of these investigations are shown in Table 5.

TABLE 5. — LOSS OF ^{32}P THROUGH THE LIFETIME OF MICE BORN FROM ACTIVE MOTHERS. MOTHER INJECTED WITH ^{32}P ON FEBRUARY 9. GESTATION: FEBRUARY 18. REPLACEMENT OF THE ACTIVE BY AN INACTIVE MOTHER: FEBRUARY 22.

No. of offspring	Killed: date	Relative activity	Weight in gm
1	22/2	100	3
2	3/3	82	7
3	16/3	73	15
4	30/3	48	18
5	13/4	41	25
6	13/5	40	35

* Incl. C^{32} of 3 offspring.

The fact that a very appreciable percentage of the maternal phosphate is preserved — though less than of the maternal calcium — is presumably due to the lower share of the bone phosphorus in the total body phosphorus than the part of bone calcium in body calcium. 17 per cent of the phosphorus content of the mouse are present in the soft tissues, but only 1 per cent of its calcium content is located there. The phosphorus and calcium atoms present in various components of the soft tissues with the exception of desoxyribo nucleic acid phosphorus of some tissues—are poorly conserved and, consequently, maternal calcium may be expected to be better conserved than maternal phosphorus.

From the fact that during the first 40 days of life—thus during a phase of intense skeleton formation—only less than a third of the maternal calcium atoms of the mouse is lost, we can conclude that the largest part of the calcium atoms leaving the circulation is utilized to skeleton formation and remains largely conserved in the skeleton.

LEBLOND and assoc.[9] injected labelled phosphate into newborn rats and followed the ^{32}P uptake by the humerus and the lower jaw. Denoting the total ^{32}P taken up by the humerus in the course of the first hour by 100, the uptake after eight hours was found to be 150, after one day 117, and after three days 116. In spite of the rapid growth of the humerus, the ^{32}P present after the lapse of a day is thus conserved through the following days; similar results were obtained in investigations on the ^{32}P uptake by the lower jaw.

The incorporation of calcium atoms in the rapidly growing bone tissue can also be studied by following its uptake into the incisor of outgrown animals. CARLSON[23, 28, 35a] performed extensive and highly instructive studies on the calcium metabolism of outgrown rats, among others with the result that the calcium atoms incorporated with the rapidly growing incisors are conserved to a very large extent in contrast to those incorporated with the outgrown skeleton.

It is rather difficult to determine the calcium intake and excretion by the suckling mouse. Our adult mice (36—37 gm), however, were found daily to consume 4 ± 0.6 gm of standard bread containing 8.3 ± 1.2 mgm. calcium; further 0.2 mgm calcium was contained in the 4 ml. of daily consumed water. The calcium recovered daily in the faeces amounted to 8 mgm. A very appreciable part of the faeces calcium may be assumed to be of endogenous origin, thus having passed the circulation before excretion. The share of endogenous phosphorus in the faeces phosphorus was calculated from the specific activity of faces P and urine (plasma) P[44, 45] these calculations lead to the result that 74 per cent of the phosphorus of the human food and 72 per cent of the rat food are absorbed into the circulation. About the same percentage of the food P can be expected to be taken up by the mouse. As to the utilization of calcium, data are available only for the uptake by humans[43].

Here, the mean percentage uptake was found to be 56. From the above data it follows that, out of the daily uptake of 8 mgm calcium by our mice, at least 4 mgm. have passed the circulation, representing a minimum amount of 2 mgm in the course of 500 days. From our results it thus follows that these 2 mgm were prevented from interchanging with $^2/_3$ of the 370 mgm calcium present in the skeleton of a mouse weighing 36 gm. The protected part of the skeleton calcium did not come into contact with the plasma or lymph and, correspondingly, an exchange between the unlabelled food calcium and labelled skeleton calcium could not take place; the same is true for the new-formation of the protected apatite crystals of this part of the skeleton under participation of food calcium. A possible rearrangement within the protected area would not manifest itself in our experiment.

The inaccessibility of parts of the skeleton minerals manifests itself also by the observation that radium, which like calcium is a strongly bone-seeking element, can find a life-long abode in the skeleton. The fact that a large fraction of radium administered to human subjects remains for decades in the skeleton is due presumably to the incorporation of the radium into parts of the skeleton which are covered by apatite layers and thus become inaccessible and, even if released, are incorporated again with the apatite structure. Aub and associates[46] report a case in which no decrease in the radium content of a woman was found to take place between 1934 and 1945. This woman had been administered radium in 1924.

CONSERVATION OF ANCESTORAL ATOMS

The radiocalcium atoms going over from the first generation of mice into the second (cf. p. 226) do not indicate the total amount of maternal calcium atoms passing from the mother to the offspring, since the mother is not uniformly labelled. Chemical data indicate a passage of about 1.3 per cent. Since the calcium of the second generation is uniformly labelled, the passage of the ancestoral calcium atoms from the second into the third generation is properly indicated by the radioactive tracer. About one third of the ^{45}Ca content of the second generation is lost prior to gestation, while about 0.5 per cent or less of the remainder passes into the third generation. From the calcium atoms present at birth in each generation, thus $^1/_{300}$ part or less goes over to the following generation. As our mice contained $6 \cdot 10^{21}$ calcium atoms, the eleventh generation did no longer contain a single ancestoral calcium atom.

It is of interest to compare the life cycle of the ancestoral calcium atoms of the mouse with that of easily accessible water molecules. Applying deutoriated or tritiated water as an indicator first the half

life of water molecules present in the rat was found to vary between 3.6 and 2.5 days[35, 36], that of the mouse is expected to be somewhat shorter. Thus, in the course of 165 days, all 10^{24} water molecules present at the start of the experiment in the mouse are replaced. About 4 per cent of the maternal water molecules go over to the offsprings and, from these, the second and third generations of offsprings will take up a share which depends on the age of gestation; the fourth generation, however, will hardly contain any more ancestoral water molecules.

When the rate of disappearance of labelled water was followed in the rat during a long period, which was made possible by using tritiated water as an indicator, it was observed[36] that, after the lapse of 30 days, the labelled water disappeared at an appreciably slower rate than with a half-life of 2.5 days. The controlling factor of the dissappearance of labelled water from the organism is now the release of firmly bound tissue tritium which again becomes a constituent of the water molecules. Due to this fact, it lasts 60 days until the number of labelled water molecules of this type, present in the mouse, decreases to a 10^{-2}th of its initial value.

If we disregard those water molecules whose hydrogen atoms were temporarily incorporated in tissue constituents and released appreciably later to become constituents of water molecules again, then all ancestoral water molecules are lost by the mouse during two generations.

While the loss of ancestoral calcium is determined mainly by the loss at birth, many ancestoral water molecules are lost during the lifetime of a generation, none of them reaching the third generation of offsprings.

Summary

Since it was desirable to obtain uniform labelling of all calcium present in the skeleton of the mouse, $^{45}CaCl_2$ was added to all water administered to mice for weeks before and after gestation. Such water was also given to new-born mice after weaning until adult age was reached. The members of the litter, having almost the same radiocalcium content, were then sacrificed at different dates within 560 days.

From the labelled calcium atoms present in the skeleton of the outgrown mice, 67.2 ± 7.9 per cent were found still to be present in the skeleton of sister mice sacrificed after the lapse of 390 days.

When administration of ^{45}Ca was interrupted after the birth of the litter, and its members reared by inactive mothers were sacrificed at different dates within 560 days, a mouse killed shortly after birth contained 8 per cent of the maternal ^{45}Ca atoms, another mouse killed after 510 days contained 4 per cent. Half of the calcium atoms present at birth is thus conserved during the lifetime of the mouse.

From the figures obtained of the passage of labelled calcium from one generation to the next, it follows that the eleventh generation does not contain a single calcium atom present in the first generation of its ancestors.

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25. PATH OF ATOMS THROUGH GENERATIONS

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The number of atoms inherited from the mother which is passed on to the next generation depends on three factors: (i) the fraction of the total number of atoms of the mother which were passed on to the newly born; (ii) the number of inherited maternal atoms that are replaced before birth of the grandchild by atoms from nutrients; and (iii) the number of maternal atoms still present at pregnancy which can be used for development of the embryo of the next generation.

The quantity mentioned first depends essentially on the weight ratio between the mother and the newly born. Sodium, chlorine, and sulfur are preferred by the body of the newly born, whereas potassium, calcium, magnesium, and phosphorus are preferred by the body of the mother. The greatest discrepancy from an equilibrium distribution is seen in calcium. The concentration of calcium is about 3 times larger in the body of the mother than in the body of the newly born. The reason for this is the calcium deficiency of the developing skeleton of the newly born.

The loss of inherited atoms before reproduction of the animal and the stability of the still-present, inherited atoms for the development of the embryo of the next generation vary decidedly from element to element.

It has long been recognized that food supplied to the body is not only used to supply energy but is also of great importance in the replacement of used parts of the body. However, only in the last decades has interest begun to develop in the quantitative side of the second function of the nutrients and in determining the lifetime of molecules and atoms in the bodies of animals and plants.

REPLACEMENT OF SODIUM ATOMS OF THE BODY

As the first example for the replacement of atoms present in the body by atoms absorbed from food, we shall consider the fate of the sodium atoms in the organism. If radioactively labelled sodium—for example, in sodium chloride—is supplied to the body, the supplied sodium ions

mix rapidly with the sodium ions circulating in the body fluid. Therefore, the percentage of elimination of radioactive sodium is equal to the percentage of elimination of the total amount of circulating and easily exchangeable bound sodium in the body.

Each day about 4 percent of the radioactive sodium present in the human body is eliminated (1—3). Therefore, the half-life of the sodium atoms circulating in the body is about 2 weeks. About 54 grams of sodium are present in the extracellular fluid of an 80-kilogram person. To this must be added an intracellular amount of about 33 grams. Of the latter, about four-fifths is contained in the mineral constituents of the skeleton (4). The intracellular sodium and part of the mineral skeleton sodium enters comparatively fast into exchange with the circulating extracellular sodium. Two-thirds of the mineral skeleton sodium, about 18 grams, is anchored so strongly that it is retained to a large extent throughout life (4—7). Of the sodium that is not strongly anchored—a total of 70 grams (2×10^{24} sodium atoms)—not a single atom is present in the body after 162 weeks. These atoms are all replaced by sodium atoms from the food.

All the chief ingredients of the body, with the exception of hydrogen, nitrogen, sulfur and iron, take part in the build-up of the apatite in the bone. The amount of conservation of a part of the individual atoms of an element in the body for a long or very long time depends foremost on the factor of how much of this elements enters into the bone apatite and is retained there temporarily or finaly.

The evaluation of the degree and the speed with which atoms of the mineral bone skeleton are replaced by atoms of the blood fluid or the lymph is therefore of the greatest importance for our problem.

EXCHANGE OF PHOSPHORUS ATOMS OF THE SKELETON STRUCTURE

The question of whether and to what degree the atoms of the skeleton structure are exchanged with those of the blood and the lymph was raised only after radioactive phosphorus became available as an indicator for phosphorus atoms. Immediately after the discovery of artificial radioactivity by Frédéric and Irene Joliot-Curie, radioactive phosphate was produced. This was first used to answer the question of whether an exchange takes place between the phosphate ions of the bone and those of the blood (8). A few minutes after radioactive phosphate had been given to an adult rat it could be traced in the bone structure. The content of phosphorus-32 in the bone skeleton increased very rapidly at first; after 1 hour, however, the increase was much slower.

The obvious interpretation of these observations was that a rapid exchange takes place between the marked phosphate ions of the fluid

blood and the phosphate ions lying on the surface of the apatite crystals of which the bone structure is built. Hand in hand with this exchange goes a biological recrystallization of the skeleton. Crystals of the bone structure go into solution, and the new crystals, which have been formed from the labelled fluid blood, have to be radioactive. The biological recrystallization—that is, the renewal of the bone skeleton—governs

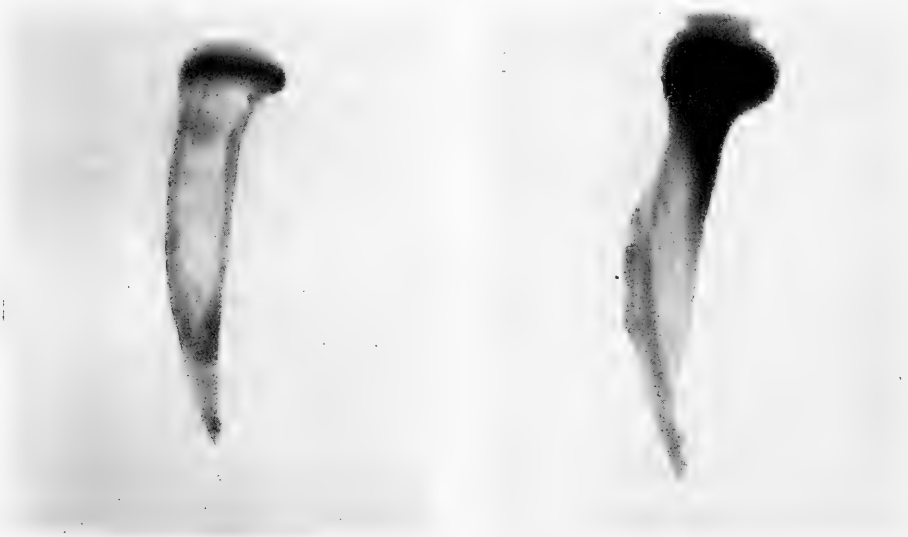

FIG. 1. Phosphorus-32 absorption by the tibia of an adult rat 5 minutes after intravenous injection (left) and 120 minutes after intravenous injection (right). (×3) [Photos courtesy of C. P. LEBLOND

this exchange process nearly completely after a short time. Autoradiographs taken by LEBLOND and co-workers (9) illustrate clearly that radioactive phosphate is absorbed by the epiphysial plate of the adult rat 5 minutes after injection (compare Fig. 1). After 2 hours, the absorption is very distinct.

It follows from the classical investigations of PANETH (10) that the ions in the topmost molecular layer fraction of a crystal powder enter into an exchange equilibrium with the ions of a surrounding solution. This statement holds only for a part of the ions which are located in the topmost molecular layer of a well-developed crystal surface—of a mineral, for example. Even if only a small of the topmost molecular layer of the bone apatite took part in the exchange proceedings, it would be sufficient for the removal of an important part of the phosphorus-32 from the plasma which was added to the blood fluid. Three percent of the bone phosphate, or maybe even more, settles in the topmost molecular layer of the bone apatite, and the phosphorus content of the latter is about 700 times greater than that of the blood plasma. If after a while

1 milligram of the phosphate of the bone skeleton shows the same radioactivity as 1 milligram of phosphate of the blood fluid, it could be concluded that the total phosphate content of the bone skeleton was renewed during the experiment. This, however, is not all the case. The experimental conditions for determining the part of the bone skeleton which is renewed are very unfavourable. The specific activity of the plasma phosphate falls at first very rapidly, later on very slowly, and shows fluctuations during the necessarily long duration of the experiment. In order to simplify the conditions for the experiment, the specific activity (activity per milligram) of the bound phosphate of a rabbit was held constant for 50 days by repeated daily injections.

It followed that about one-third of the soft epiphysial bone skeleton was renewed during the duration of the experiment (11). From the hard diaphysial bone structure, a considerably smaller fraction is renewed. The exchangeable part of the bone structure of the rabbit has therefore to be limited to about one-third.

FATE OF CALCIUM ATOMS

In order to be able to pursue the fate of calcium through several generations of mice, we had to know what fraction of the calcium ion in the bone skeleton of mice is renewed during a lifetime. Numerous experiments concerning the calcium metabolism of the skeleton have been described (12—16). In determining the fraction of the exchangeable skeleton calcium, we continued the afore-mentioned experiments, and we replaced the very tedious method by another. We did not investigate the replacement of the inactive bone calcium by labelled calcium in the fluid blood. We bred animals that were evenly marked with radioactive calcium, calcium-45. We determined then the fraction of radioactive calcium ions that left the animal during its lifetime and that were replaced by inactive calcium ions from the food. We supplied the mother with radioactive calcium of limited activity (less than 0.5 microcurie) so that no radiation damage to the animal had to be feared. In this way we obtained an evenly activated generation of mice, which cannot be obtained by any other method. Each member of a single generation had the same calcium-45 content within a few percent. The mice received radioactive calcium with their food till they were grown up. One of the siblings was killed at birth, and the radioactivity of its skeleton was determined. The remaining animals were killed at other times and examined. It became evident that after more than 1 year, which represents a considerable part of the life span of a mouse, 6.7 ± 7.9 percent of the original calcium atoms of the skeleton could still be found. Therefore only one-third of the bone skeleton is renewed.

In another series of experiments we examined newly born mice right after birth. The other mice were transferred to an inactive foster-mother and analyzed at different times in the following 1.5 years. Figure 2 shows the results of some of these experiments. One should expect that during the intense growth of the first week of life a large part of the atoms taken over from the mother are disposed of and replaced by those from

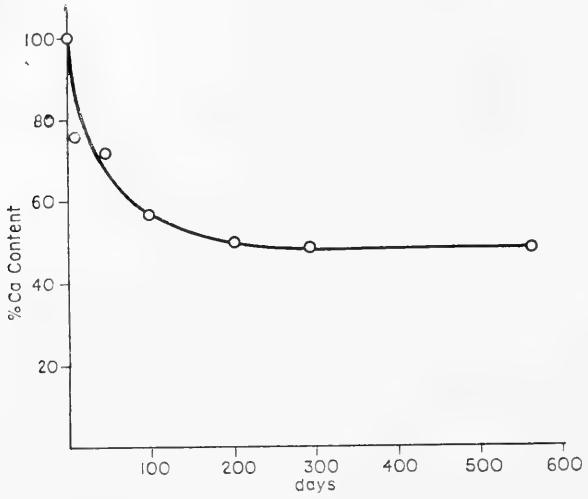

Fig. 2. Loss of inherited calcium atoms during the life of a mouse. We determined the total activity of siblings whose mother was fed calcium-45. The offspring were killed at different times

food. In the first 50 days, the loss of atoms from the mother is considerable. However, in the whole life of a mouse it does not amount to more than 50 percent.

We found about 1/300 of the labelled calcium atoms that the mother of the second generation received at its own birth, in the newly born of the third generation. The loss in calcium atoms of the ancestors before propagation and through limited transmission to the offspring is repeated from generation to generation. It can therefore be found by extrapolation that, of the 6×10^{21} calcium atoms to be found in a mouse weighing 30 grams, not one is present in the 11th generation of its descendants. The loss of labelled calcium atoms in the transition from the second to the third generation of one strain of mice amounted to 1/200 only. Calcium atoms from the mother should no longer be traceable in the 12th generation of these animals.

That the calcium atoms of the ancestors can be traced in such a long series of descendants can be ascribed to the fact that about 99 percent

of the body calcium is located in the skeleton. The skeleton is therefore able to preserve an important part of its building materials. However, only a fraction of this calcium is available for the structure of the body of the descendants.

FATE OF WATER MOLECULES

The calcium and phosphorus atoms of the ancestors of the mouse (similar conditions should be valid for human beings) are traceable in a long series of generations in the descendants. However, the water molecules that the mother transmits to the descendants disappear during the life of the first generation.

Shortly after the discovery of heavy water, we were able to determine, thanks to the support of H. C. Urey, who discovered this isotope, the half-life of water molecules in the human body. We found that this amounts to about 10 days in the body (17) for normal water intake. The half-life depends on the amount of water consumed. Schloerb and his co-workers (18) also found recently a half-life of 10 days with a normal daily intake of 2.7 liters. In the case of a rather large daily water intake of 12.8 liters, the lifetime fell, however, to 2.5 days. In the case of a normal water intake, after 810 days not a single molecule of the 2×10^{27} water molecules originally present remains in the human body.

The half-life of water molecules in rats was determined to be 2.5 to 3.5 days. In the mouse it should amount to about 2.5 days. After 340 days, therefore, not a single maternal water molecule is present in the mouse.

Owing to the large part that the element calcium plays in the development of the skeleton, it is preserved best in the descendants. However, even of this element, not a single atom of the ancestors is present in the 11th and 12th generations. This evidence illustrates the independence of the hereditary pattern from an atomic share of the forefathers. It is well-known that the hereditary pattern depends on the ability of the organism to group in atoms, molecules, and higher cellular units in a certain manner. A protein composed of 20 different amino acids and having a molecular weight of 100,000 is able to appear in more than 10^{1270} isomers, as calculated by Staudinger (19). Therefore, incomparably more types of proteins can exist than the number of water molecules (10^{46}) which are present in the oceans of the world. Since hereditary patterns are tied to the reproducibility of individual proteins or nucleoproteins, there is room for new individual hereditary patterns as long as the number of human beings has not reached 10^{1270} or even a larger figure.

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26. NOTE ON THE CHLORIDE CONTENT OF THE MINERAL CONSTITUENTS OF THE SKELETON

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In view of the only slightly differing size of the hydroxyl and fluoride ion, the hydroxyl ions of the bone apatite can be replaced by fluoride ions. Furthermore some of the fluoride may be present in the bone mineral as calcium fluoride.

The fluoride content of the bone apatite is determined by that of the plasma, which in turn depends on the fluoride content of the food. The fluoride content of the earth's crust is much lower than that of the seawater, the mineral constituents of the skeleton of mammals living in the sea is, correspondingly, very much, about eleven times, larger than that of mammals living on land, which contain about 0.05 % fluoride only. The skeleton of fish living in the Baltic, which has a low fluoride content, have a much lower fluoride percentage (0.06 %) than the skeleton of fish living in the Atlantic (0.43 %). Incorporation of fluoride into the mineral constituents of the bone was in recent years much investigated, mainly in connection with the observation that the presence of fluoride in the mineral constituents of teeth increases their resistance to caries.

The radius of the chloride ion is much larger (1.81 Å) than that of the fluoride ion (1.33 Å) and calcium chloride being very soluble we can expect to find slight amounts of chloride in the mineral constituents of the bone. While the X-ray diagram of fluoroapatite is almost identical with that of hydroxy-apatite that is far from being the case for chloroapatite[9]. The fluids circulating in the bone tissue having a high chloride content (about 300 mgm/100 ml); this has to be quantitatively removed prior to the determination of the amount of chloride incorporated into the mineral constituents. Such removal by chemical treatment of the bone encounters great difficulties. However, when labelling the skeleton all through with radiochloride and placing the animal, *e. g.* the mouse, on diet containing non-radioactive chloride for several months, all exchangeable radiochloride will be removed and excreted, the radiochloride fixed in the mineral constituents alone remaining in the skeleton.

If the human half of the exchangeable chloride is removed in the course of 14 days[1] and replaced by chloride of the food and after 6 months, *i.e.* after 12 periods, all exchangeable chloride initially present will be practically absent. In the mouse, with its high metabolic rate, the removal rate of chloride can be expected to be still higher than in man. The removal can be accelerated by increased chloride feeding. Besides the chloride present in the standard biscuits fed we added 0.2% NaCl to the water the mice were drinking after they were put on non-radioactive diet. To the water administered to the mice in the first phase of the experiment ^{36}Cl of 0.67 μ C activity per liter was added as sodium chloride weighing 9.3 mgm. The labelled chloride was administered to pregnant mice about 2 weeks prior to gestation. After gestation the administration of labelled chloride was continued for 4 months when the animals were fully grown. One member of each litter was then killed and its total ^{36}Cl content and that of its skeleton determined. The remaining members of the litters were investigated 6 months later.

The bone was ashed in the presence of sodium carbonate. The activity of 100 mgm, thus of an infinite thick layer of the samples obtained was determined, the counts registered being multiplied by the total weight of the ash-sodium carbonate mixture.

The total ash of the first member of the litter of the mice investigated had a total activity of 2780 counts per min, mean value 2910 ± 452 counts, that of the the mineral constituens of the skeleton of the first investigated offspring 56.6 counts. This was prior to biological removal of all exchangeable chloride from the skeleton but after the removal of some of the latter in the course of the isolation of the mineral constituents.

By keeping the mice on an activity-free diet for 6 months the activity of the mineral constituents declined in the average to 21.2 counts (cf. Table 1).

The total chloride content of a 35 gm mouse taken to be 48 mgm, the activity of 1 mgm of the body chloride prior to removal of the active food was 60.6 counts per min. As the total skeleton after biological removal of all exchangeable chloride had an activity of 21.2 counts per min. the sequestered chloride content of the bone mineral amounted to 0.35 mgm or 0 73% of the total body chloride.

The sequestered fraction of the bone calcium of the mouse, that non-replaceable by circulating calcium, was found[2] to be $67.2 \pm 7.9\%$. The corresponding figure for bone sodium is stated to be 60—70[3], 65[4], 60[5] and 69[6] by different authors. Thus about a similar percentage of excess sodium and of excess calcium is prevented from interchanging with their circulating atoms. The sequestration of bone constituents is presumably due to the fact that a contact between these constituents and the circulating body fluids is obstructed. To arrive at the total

excess chloride content of the bone of the mouse we correspondingly have to multiply the figure of 0.35 mgm found for the non-exchangeable bone chloride by about 1.5, thus arriving at the result that the total excess bone chloride content of our 35 gm mice amounts to about 0.53 mgm.

TABLE 1. — COUNTS PER MIN. ^{36}Cl ACTIVITY OF THE SKELETON OF 33—36 G MICE AFTER BEING KEPT ON NON-ACTIVE DIET, THUS AFTER BIOLOGICAL REMOVAL OF EXCHANGEABLE RADIOCHLORIDE, FOR 6 MONTHS

18.2
24.6
25.1
28.6
25.1
26.6
25.5
16.4
19.4
12.1
211.6 : 10 = 21.2 ± 2.08
m.eV.: = ± 0.208

In contrast to the bone sodium which is to a large extent present as excess sodium in the skeleton it can be shown that the chloride present as excess chloride makes out 1/10 only of the total bone chloride.

From 234 m-equiv. sodium present in 1 kgm of dry human bone 84.9%, thus 46 gm, was found by Edelman *et al.*[5] to be excess sodium in a 70 kgm man. For the dog Edelman and associates found 89.5% of the bone sodium to be excess sodium and a similar figure is stated by Miller and associates[7]. 1 kgm of rat bone was found to contain 125 m-equiv excess sodium[8]. As to the total chloride content of 1 kgm fat free bone this was found to amount to 19 m-equiv. only[5], thus to less than the extracellular bone sodium which makes out 25 m-equiv. While excess bone sodium is to a marked extent responsible for the difference between total and extracellular body sodium, for chloride this difference is almost entirely due to the presence of intracellular chloride in the soft tissues.

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Comment on papers 24, 25, 26

The availability of ^{45}Ca at a later date much facilitated the investigations of processes taking place in the bone apatite. Experiments taking years could be carried out, which had not been possible before. Furthermore, in contrast to the body phosphorus, which is only partly found in the skeleton, 99 per cent of the body calcium of the mouse is concentrated in the latter. In paper 24 experiments are described in which ^{45}Ca was administered to pregnant mice and also to the offsprings until they were fully grown. After that date, they were kept on a non-radioactive diet. Mice were then killed at intervals, and the ^{45}Ca content of their skeleton determined. In the course of 2 years, which covers the largest part of the life span of the mouse, 33 per cent of the skeleton ^{45}Ca, and thus of the skeleton calcium, was found to be replaced by calcium atoms of the food or present formerly in the soft tissue. When ^{45}Ca was administered to the pregnant mouse alone, the offspring were found to conserve 50 per cent of the maternal calcium atoms through life. When similar experiments were carried out with ^{32}P (paper 20) 60 per cent of the ^{32}P acquired by the mouse at birth was found to be lost when reaching maturity (paper 23). The calcium atoms have a fairly stable position in the skeleton so that it takes a great number of generations until the last calcium atom originating from the first ancestor is lost. In contrast with calcium atoms, the last ancestoral water molecule is lost already after a few generations, as described in paper 25. The above-mentioned renewal figures indicate replacement of skeleton calcium atoms by such taken up with the food or formerly located in soft tissues. Changes in the apatite crystals without participation of food calcium or soft tissue calcium would not be indicated by the methods described.

In the course of a symposium which took place in the Ciba Foundation in 1951, the question was raised of how much chloride is to be found in the mineral constituents of the skeleton. This question, which could not then be answered, induced an investigation the results of which are found stated in paper 26. The non-exchangeable chloride fraction was found to remain very much behind the non-exchangeable sodium fraction of the mineral constituents of the bone.

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27. THE FORMATION OF PHOSPHATIDES IN THE BRAIN TISSUE OF ADULT ANIMALS

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It is generally assumed that no regeneration of the brain tissue of adult animals takes place. To test the validity of this assumption we investigated whether any formation of phosphatides takes place in the brain tissue of adult animals. This problem cannot be attacked by ordinary chemical methods because these do not permit the making of a distinction between phosphatide molecules formed at different dates; this is, however, possible if we introduce a labelled phosphate into the animal body (Chievitz and Hevesy, 1935, 1937), labelled sodium phosphate for example, and investigate whether the formation of labelled phosphatides can be established in the brain of the animal. We carried out experiments on rats, mice, and rabbits.

Labelled phosphorus can be obtained by adding radioactive phosphorus to "normal" phosphorus. If we dissolve for example 1000 relative (radioactive) units of the radioactive phosphorus isotope ^{32}P in a solution containing 1 mgm of phosphorus, say as sodium phosphate, and administer this solution to an animal, then the presence of 1 relative radioactive unit in a part of the animal tissue will prove the presence of $^{1}/_{1000}$ of the total number of phosphorus atoms administered. The radioactive ^{32}P used in our experiments was obtained by bombarding carbon disulphide with fast neutrons from a mixture of radium sulphate and beryllium.

Phosphatides are the second most abundant constituents of the brain tissue. The composition of the latter is shown in Table 1.

Table 1. — Composition of the dry Brain Tissue of Adult Albino Rats

	%
Protein	48.5
Phosphatides	26.5
Lipides not containing P	15.2
Esters and inorganic constituents	9.8

The total phosphorus present, amounting to 1.39%, is distributed between protein, phosphatides and acid soluble compounds in the following manner.

Protein P	6.8%
Phosphatide P	67.6%
Acid soluble P	25.6%

the largest amount of phosphorus being thus present as phosphatide. Let us assume that labelled sodium phosphate is introduced per os or by injection into the animal body and that after the lapse of some time some of the inorganic phosphorus present in the brain tissue is found to be labelled. Such an observation is not to be interpreted as a formation of new brain tissue because the inactive phosphate ions can be replaced by labelled ones through a simple exchange process. The phosphate present in the lecithin molecule cannot, however, be replaced through a simple exchange process i.e. the labelled phosphate can only enter the lecithin molecule during a synthesis of the latter. The presence of labelled lecithin molecules in the brain is therefore a proof that a synthesis of lecithin has taken place after the introduction of the labelled sodium phosphate into the animal body. Though it was highly improbable that the phosphate present in the lecithin-molecule could be replaced by labelled phosphate through a physical exchange process, we tested this point by carrying out the following experiment. We shook 10 cc. of cats blood at 37° for 4 hours with 2.5 cc. of isotonic sodium chloride solution containing labelled phosphorus. 10 cc. of blood contain about 1.2 mgm of phosphatide and 0.4 mgm of inorganic phosphorus, the latter being labelled by the activity added. The lecithin was then extracted. The extraction was made with ether + alcohol and the extract was shaken for several hours with calcium phosphate to remove any inorganic labelled phosphate which might be present in the extract. Of the 10,000 radioactive units only 3 units were found in the blood phosphatide extracted. Even this very slight exchange is probably due to enzymatic actions occurring in the blood. From an experimental point of view blood seemed to be a very suitable liquid to carry out an exchange experiment and as the exchange observed was only a very slight one it did not seem of interest to pursue the subject further and carry out exchange experiments in liquids from which enzymes had been removed. In this connection we may, however, mention an experiment in which blood containing labelled phosphate was allowed to circulate through an isolated liver.

Professor Lundsgaard being engaged on liver perfusion experiments kindly added a solution (3 cc.) containing labelled phosphorus of negligible weight to the blood used in his experiments. In 10 cc.

of blood used for 4 hours, 7 of the 10,000 radioactive units added were found in the isolated lecithin phosphorus; the liver perfusion has thus a positive effect on the formation of labelled lecithin.

We may also mention that in 1 cc. of the blood of a cat killed $1\frac{1}{2}$ hours after injecting a negligible weight of labelled phosphorus, we found a lecithin phosphorus activity amounting to 2% of the activity found in 1 cc. of plasma, while the acid soluble phosphorus present in 1 cc. of blood corpuscles contained, as Professor LUNDSGAARD found, an activity of 12.5% of that of the plasma, about $\frac{2}{3}$ of this being present in the phosphorus esters. A detailed study of the distribution of the labelled phosphorus between plasma and blood corpuscles is being carried out by Professor LUNDSGAARD and one of the present writers.

EXTRACTION OF PHOSPHATIDES

The usual method of extracting phosphatides is by means of alcohol-ether mixtures. In this method of extraction a small part of the inorganic phosphorus present is dissolved as well; but in view of the preponderance of phosphatide phosphorus in the brain the error thus introduced can generally be disregarded. Under the peculiar conditions which prevail in the investigation of the formation of labelled phosphatides the error mentioned above can however become very embarassing. We introduce labelled inorganic phosphorus into the animal body per os or by subcutaneous injection. Now it is possible that only a very small amount of this is converted into labelled phosphatide phosphorus so that even of a trace of the labelled inorganic phosphorus is extracted by the alcohol-ether mixture, our results can be seriously falsified. This is best seen from the following example: brain tissue is shaken *in vitro* with a solution of labelled inorganic phosphorus containing 10,000 relative radioactive units, and 0.1 mgm phosphorus; the solution is then removed and the dried tissue extracted with alcohol + ether. The presence of as little as 10^{-4} mgm of inorganic phosphorus in the extract corresponds to 10 relative radioactive units and may be partly or wholly responsible for the activity of the extract. For this reason, although conditions in experiments *in vivo* are much more favourable than those in the example above, we chose a method of extraction more suitable for our special case than the alcohol-ether extraction.

The procedure adopted by us was as follows. We dried the brain tissue with acetone and extracted the phosphatides by prolonged shaking with carefully dried ether; the extract obtained was evaporated to dryness and dissolved a second time in ether in the presence of a large excess of finely powdered dry sodium phosphate. We had found that

by shaking a solution of labelled sodium phosphate with a large excess of finely powdered unlabelled sodium phosphate the former can be removed from the solution. A distribution of the phosphate ions between the liquid and solid phase takes place and the chance of a labelled phosphate ion being in solution is entirely negligible on account of the overwhelming excess of the solid phase; this is especially so when the whole procedure is repeated. Another method of purification of the ether extract from the labelled inorganic phosphorus was as follows. Unlabelled sodium phosphate was dissolved in the extract and precipitated as ammonium magnesium phosphate. By repeating this procedure it was possible to get rid of the slightest trace of labelled inorganic phosphorus present in the ether extract. The activity of the ether extract had then to be measured. The amount of material being small (2.3 mgm) it was advisable to add a carrier. An inactive commercial lecithin preparation was used for this purpose, some of it being dissolved in the ethereal solution before evaporation. Before destroying the lecithin, calcium oxide was added to bring about the formation of calcium phosphate. The activity of the latter was measured by means of a Geiger—Müller Counter.

INVESTIGATION OF THE BRAIN OF RATS

All our investigations were carried out on fully-grown adult rats. One animal was killed after a lapse of 5 days, a second one after a lapse of 3 days, and the third one after one hour. The results obtained are seen in Table 2.

TABLE 2

Rat killed after	Fresh weight of brain in mgm	Dry weight of brain in mgm	Percentage of labelled P found	
			in the brain	in brain phosphatide
5 days	1800	380	$7 \cdot 10^{-2}$	$3.7 \cdot 10^{-2}$
3 „	1440	300	$7 \cdot 10^{-2}$	$2.4 \cdot 10^{-2}$
$^1/_{24}$ „	1430	290	$6.8 \cdot 10^{-2}$	$0.42 \cdot 10^{-2}$

The above figures show clearly the formation of labelled phosphatide in the brain of adult animals. Though it could hardly be doubted that what we extracted and tested was actually phosphatide we obtained further evidence of this preparing from the brain tissue of rats to which labelled phosphorus had been administered, the highly characteristic chlorocadmium compound of lecithin and tested its activity.

PREPARATION OF CHLOROCADMIUM LECITHIN

The ether extract of the brain was evaporated to dryness, the residue was dissolved in alcohol, and a saturated solution of cadmium chloride in methyl alcohol added. The chlorocadmium lecithin which precipitates from the solution was further purified in the following manner: the precipitate was suspended in chloroform and a solution of ammonia in methyl alcohol added, whereupon the lecithin remained in solution while the cadmium is precipitated. The next step was to evaporate down the solution containing the lecithin, and to dissolve the latter in alcohol; the whole purification process was then repeated. We ascertained in our preliminary experiments that the above lenghty process can be carried out with a yield of about 50%. In an experiment with a rat's brain extract which showed an activity of $2.4 \cdot 10^{-2}$ % of the total amount given to the animal, we recovered, after the preparation of the chlorocadmium compound, lecithin containing $1.2 \cdot 10^{-2}$ % of the total activity administered.

INVESTIGATION OF THE BRAIN OF MICE

As has been mentioned already it is of great importance to investigate the brains of animals no longer growing. Through the kindness of Professor KROGH and Dr. HAGEDORN we obtained mice of unusually high age and investigated their brain. The animal was killed 21 days after injecting the labelled phosphorus. A result obtained was the following:

TABLE 3

Weight of fresh brain	Weight of dry brain	Percentage of labelled phosphorus administered found	
		in the brain	in brain lecithin
814 mgm	86.2 mgm	$1.35 \cdot 10^{-1}$	$5.5 \cdot 10^{-2}$

INVESTIGATION OF THE BRAIN OF RABBITS

The investigation of a brain of a rabbit killed 27 days (I.), resp. 4.5 hours (II.) after injection with radioactive phosphorus gave the following result.

TABLE 4

Weight of fresh brain	Weight of dry brain	Percentage of labelled phosphorus administered found	
		in the brain	in brain lecithin
I 8940 mgm	1400 mgm	$6.9 \cdot 10^{-2}$	$2.3 \cdot 10^{-2}$
II		$1.8 \cdot 10^{-3}$	$6.3 \cdot 10^{-3}$

RELATIVE ABUNDANCE OF LABELLED PHOSPHORUS IN THE BRAIN COMPARED WITH THE AMOUNT PRESENT IN OTHER ORGANS

The relative abundance of labelled phosphorus in different organs (activity per mgm phosphorus) is seen from Table 5.

TABLE 5

Animal	Killed after	Total Brain (A)	Brain Lecithin (B)	$\frac{A}{B}$	Bone
Rat	5 days	2.12	2.34	0.9	1 (tibia)
Rat	3 days	2.12	2.10	1.0	1 ,,
Rat	1 hour	2.12	0.40	5.3	1 ,,
Mouse	21 days	0.94	1.08	0.9	1 ,,
Rabbit I	27 days	6.10	6.10	1.0	1—3.5*

* 1 = Tibia diaphysis, 2.6 = Jaw, 3.5 = Tibia epiphysis.

As is seen from the above figures the ratio between labelled phosphorus in the whole brain and labelled phosphorus in brain lecithin shifts in favour of the latter with increasing time; furthermore that the percentage replacement of phosphorus atoms in the brain by labelled phosphorus atoms is not very different from that found in the case of the phosphorus atoms of the bone.

NOTE ADDED IN PROOF: Professors ARTOM, PERRIER, SARSANA, SANTAGELLO and SEGRÉ most kindly sent us a manuscript of a paper in which the formation of lipidic phosphorus in different organs has been demonstrated by using radioactive phosphorus as indicator. They found the most marked metabolism in the liver, the intestinal mucosa and the kidney, the least in the brain and the muscles.

WEIGHT OF THE NEWLY FORMED LECITHIN

So far we have only calculated lecithin phosphorus formed as a percentage fraction of the total activity given to the animal. In what follows, we will try to calculate the amount of newly formed labelled lecithin in grams. This is a more difficult problem. If we inject 1 mgm of phosphorus showing an activity of 1000 units we know that the presence of 1 unit of activity indicates $^1/_{1000}$ mgm of the phosphorus atoms injected. Now in the blood plasma of a rat we have for 17 cc.

of blood with an inorganic phosphorus content of 4.5 mgm per 100 cc., 0.75 mgm of phosphorus; if we inject radioactive phosphorus (1000 units) of negligible weight, 1 activity unit will indicate 0.75/1000 mgm of the phosphorus originally present in the plasma. In the blood plasma, however, numerous fast processes take place in which the phosphorus atoms are involved; this is shown by investigations carried out with labelled P. We get beside other reactions, a partition of the phosphate ions between the plasma and the phosphate in the skeleton, and in view of the very large preponderance of the latter a large part of the labelled phosphate ions will soon be present in the skeleton. Similar considerations apply to the muscles and other organs. The comparatively large amount of phosphorus ester present in the blood corpuscles will also take part in a dynamic interchange with the labelled phosphorus atoms present in the plasma. In the course of the last two years Prof. E. LUNDSGAARD and one of us have carried out and extended investigation of this point, to be published shortly. As a result of the processes just mentioned, only a few, let us say 10, units remain behind of the 1000 activity units introduced into the plasma, while the total inorganic phosphorus content of the plasma is unchanged and amounts as before to 0.75 mgm. The effect will be that 1 activity unit will now indicate as much as 0.75/10 mgm (i.e. much more) phosphorus. On account of the rapid dynamic happenings in the animal body our scale of indication will rapidly change and, the function representing this change being a very intricate one. One way to obtain some information is the following. We make an experiment of very short duration in which 1 activity unit does actually indicate 0.75/1000 mgm of labelled phosphorus; then we make a longer experiment and determine experimentally the activity of the inorganic phosphorus present in the plasma. If only 10 activity units are now present then 1 activity unit indicates 0.75/10 mgm P at the latter stage. The activity accumulated at the early stage will be correctly interpreted by making use of the first mentioned scale, that accumulated at the last stage by making use of the last mentioned scale. In some cases we have followed the labelled P content of the blood continuously. Let us now consider the rat killed after 1 hour, the blood plasma of the animal containing 0.75 mgm inorganic phosphorus, a $4.2 \cdot 10^{-3}$th part i.e. $3.2 \cdot 10^{-3}$ mgm of this was converted into brain lecithin phosphorus. This being a very small part of the brain lecithin phosphorus, the reverse reaction can be disregarded and the amount of labelled brain lecithin phosphorus formed in 100 hours can be taken as a hundred times as much, i.e. $3.2 \cdot 10^{-1}$ mgm i.e. 8 mgm of the brain lecithin is newly formed in the course of about 4 days. The calculation of the weight of labelled phosphorus was carried out on the basis of an analysis of the activity of the blood and of the brain lecithin after the lapse of one hour. Now one part of

the labelled lecithin was already formed after the lapse of a few minutes, at which time the ratio $\frac{\text{mass of inorganic P}}{\text{activity}}$ in the blood was smaller and 1 activity unit therefore indicated a smaller mass of phosphorus than after the lapse of one hour when the measurements were actually made. Hence the actual amount of labelled lecithin phosphorus will be less than that calculated above but is definitely higher than 0.08 mgm.

PHOSPHORUS EXCHANGE IN BRAIN LECITHIN IN VITRO

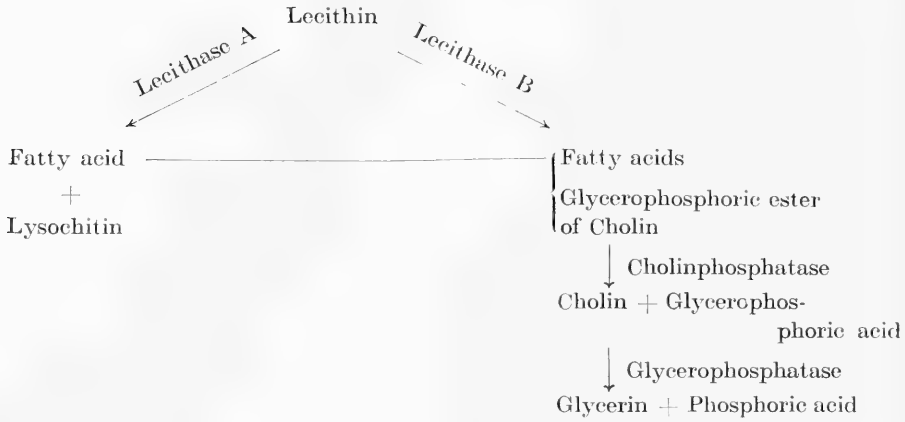
We shook a freshly removed rat brain for 5 hours at 37° with 3 cc. of an isotonic sodium chloride solution containing 0.09 mgm of phosphorus labelled by the addition of radioactive phosphorus. The brain was carefully washed with cold acetone and dried at room temperature and the lecithin extracted as described above. The ethereal solution was shaken for several hours with sodium phosphate to remove inorganic labelled phosphorus and after this operation had been repeated four times, the sodium phosphate was found to be entirely inactive. The solution showed an activity of 2200 units and we found 18 units in the lecithin extract; this corresponds to 0.00076 mgm of labelled phosphorus or about $^1/_{5000}$ part of the total lecithin phosphorus content of the brain. Thus the formation of a very small amount of labelled phosphorus also takes place in the freshly removed brain tissue *in vitro*, presumably under enzymatic action. We intend to follow up the exchange problem *in vitro* in greater detail.

DISCUSSION OF THE RESULTS

From the results above it clearly follows that brain lecithin is constantly being synthesized in the brain tissue of adult animals. Presumably a part of the lecithin is constantly broken down under enzymatic action and rebuilt again, thus making it possible for the labelled phosphorus atoms present in the blood to enter the lecithin molecule.

Belfanti, Contardi and Ercoli (1936) give the following scheme according to which lecithin is supposed to decompose under the action of lecithases.

It is possible that a reaction takes place in both directions according to this or a similar scheme, so that the phosphorus atoms present in lecithin, are rendered exchangeable when enzymes are present although they undergo no exchange in the absence of these. The study of the mode and rate of action of the different lecithases may be much facilitated by following up exchange process in lecithin and its decomposition products in the presence and absence of the different enzymes.



The figures in our experiments show clearly that the ratio of labelled lecithin phosphorus to labelled phosphorus other than that from lecithin in the brain increases with time. The amount of labelled lecithin produced within 1 hour in the brain of a rat can be estimated as lying between 0.08 and 0.0008 mgm.

In starting this research we contemplated the possibility of the formation of labelled brain lecithin being influenced by nervous action. No effect of nervous actions on chemical processes in the brain has yet been ascertained; the dependence of the latter on the former must be described by a curve where a very large increase in the abscissa (nervous action) corresponds to a minimal change in the ordinate (chemical effect). To test this possible effect of nervous action it would be necessary to carry out a very large number of experiments.

Summary

By using labelled (radioactive) phosphorus as indicator, it was found that one hour after the subcutaneous injection of labelled sodium phosphate, labelled lecithin was already formed in the brain tissue of fully grown rats. Similar experiments were also carried out with fully grown mice and rabbits. The result proves that a constant breakdown and building up of lecithin takes place in the brain tissue presumably under enzymatic action.

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28. LECITHINAEMIA FOLLOWING THE ADMINISTRATION OF FAT

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About two hours after the administration of a meal containing fat, the fat content of the blood begins to rise. Bloor[1] found that when olive oil is administered to a dog, besides an increase in the neutral fat content of the blood an increase in its lecithin content also takes place. The average increase was found to be about 20 per cent. A maximum is reached after four hours. Bloor was inclined to ascribe the lecithin formed after the administration and resorption of the neutral fat to a synthesis occurring inside the red blood corpuscles. Other explanations might, however, be suggested as well, namely: (1) The lecithin is synthesized in the intestinal mucose and resorbed into the blood. (2) The synthesis takes place, after the resorption of neutral fat, in the liver, or somewhere else outside the intestinal tract. (3) The increase in the lecithin content of the blood is due to mobilization of preformed lecithin after the resorption of the neutral fat.

To decide which of these suggestions is to be accepted we repeated Bloor's experiment, but administered simultaneously with the oil-labelled (radioactive) phosphorus in the form of sodium phosphate. In the case denoted by (1) the additional blood lecithin should contain chiefly labelled phosphorus; in case (2) the additional lecithin should contain only small amounts of labelled phosphorus; in case (3) the additional lecithin should contain ordinary phosphorus only.

We determined the normal P present in the blood lecithin, which was extracted by the usual procedure, by the method of Fiske and Subbarow, and the labelled P by means of a Geiger counter. While, as seen in the table, the lecithin phosphorus content of 100 cc. of blood increased by 2 mgm four hours after administering the oil, that of labelled P only increased by 0.096 mgm. We must, furthermore, take into account the fact that half the labelled phosphorus administered two hours before the oil produced 0.028 mgm labelled lecithin P during that time. We must therefore deduct 2×0.028 mgm from the 'oil effect' of 0.096 mgm, obtaining 0.04 mgm per 100 cc. of blood for the maximum value of the 'oil effect'.

An important objection can, however, be raised to our conclusion; it may be argued that the intestinal tract might contain large amounts of phosphorus other than the labelled phosphate administered by us, the presence of which must be accounted for when carrying out the above calculation. To investigate this point and to ascertain to what extent the labelled phosphorus was resorbed, we killed the dog after the last experiment, the results of which are seen in the table. We washed the intestinal tract with water and determined both its total P content and its labelled P content. We found by activity measurements 39.6 mgm labelled P and by chemical determination 175 mgm normal P. Within six hours as much as 259.4 mgm of the 300 mgm administered to the dog was thus resorbed. The 135 mgm. non-labelled phosphorus reached the intestine, presumably along with the digestive fluids, so that the 40 mgm labelled P were 'diluted' to 175 mgm. We determined also the total acid-soluble phosphorus content of the intestinal mucose; it was found to amount to about 40 mgm, bringing the above figures up to 215 mgm. But even if we make the assumption that this dilution was present during the whole of the resorption process we should get the result $5.2 \times 0.064 = 0.21$ mgm per cent lecithin P, while an increase of 2 mgm per cent was found in the blood lecithin P.

Time in hours	Labelled P given in mgm.	Lecithin phosphorus found in 100 cc. blood		Labelled total P found	
		Total	Labelled	in 100 cc. blood	in the total blood of the dog
0	150	—	—	—	—
2	150 (+ 50 gm oil)	16.0 mgm	0.028 mgm	1.03 mgm	6.18 mgm
4	—	15.5 ,,	0.048 ,,	2.03 ,,	12.18 ,,
6	— (259.4 mgm *resorbed*)	18.0 ,,	0.096 ,,	2.00 ,,	12.00 ,,

It is of interest to compare the labelled P content resorbed with that actually found in the blood stream of the dog. Six hours after the beginning of the experiment, as is seen in the table, only 4.6 per cent of the amount resorbed was found. This result illustrates beautifully the great rapidity of the phosphorus exchange in the body. As observed by us in numerous cases, the individual phosphorus atoms present in the blood stream exchange their places rapidly with others present in the different organs. For this reason we can conclude with certainty that during our experiments the ratio labelled phosphorus to ordinary phosphorus must have been appreciably higher in the intestinal mucose than in the blood.

The only moderate increase in labelled phosphorus in the blood lecithin after administration of oil, an increase which nevertheless in all our experiments exceeds the increase observed after the radioactive

phosphorus was administered alone, leads to the conclusion that during the absorption of neutral fat, lecithin is formed outside the intestinal tract. A comparatively rapid formation of labelled lecithin in several organs in the course of normal metabolism has in fact recently been observed[2].

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29. FORMATION OF PHOSPHATIDES IN LIVER PERFUSION EXPERIMENTS

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About 2 hr after a meal containing fat, the fat content of the blood begins to rise. This alimentary lipaemia is followed by lecithinaemia [Reicher, 1911; Bloor, 1915], the phosphatide content of the blood increasing more or less parallel with the fat content. A maximum is reached after about 4 hr and after 8 hr the fat and phosphatide contents of the blood are almost at the initial level. As to the origin of the phosphatides responsible for alimentary lipaemia the following possibilities exist:

(1) the phosphatides are synthesized in the intestinal mucosa and resorbed into the blood;

(2) they are synthesized in the blood;

(3) they are mobilized under the influx of lipaemic blood from the liver or other organs and possibly wholly or partly formed in the former during the influx.

To obtain further information on the above problem, oil together with labelled (radioactive) sodium phosphate, were administered to a dog [Hevesy and Lundsgaard, 1937]. If the increase in the phosphatide content of the blood which amounted to 15 % after 4 hr. was due to phosphatides taken up from the intestine, the phosphatides extracted from blood should have shown a marked radioactivity. The latter was, however, much smaller than to be expected on this assumption. It follows that while phosphatides are synthesized in the intestinal mucosa [Artom *et al.*, 1937; Sinclair and Smith, 1937] and some do enter the circulation from the bowels [Himmerich, 1934; Süllmann and Wilbrandt, 1934; Freeman and Joy, 1935] the bulk of the phosphatides which are responsible for the alimentary lipaemia must originate from outside the intestinal tract. We next tested the possibility that the additional phosphatides are formed in the lipaemic blood [Hahn and Hevesy, 1938]. A few ml. of dog blood were shaken with labelled sodium phosphate under the usual precautions for 4.5 hr. The phosphatides extracted after the experiment were only slightly radioactive, the labelled

phosphatides formed amounting to only about 0.1% of the total amount present. No difference was found in the behaviour of normal and lipaemic bloods.

FORMATION OF LABELLED PHOSPHATIDES IN PERFUSION EXPERIMENTS

Through the great kindness of Prof. LUNDSGAARD and Dr BLIXENCRONE, who carried out perfusion experiments on isolated livers, we were enabled to test the formation of labelled phosphatides in the blood of cats circulating through an isolated liver and also in the liver tissue. To 120—160 ml. of cat blood diluted to about twice its volume with physiological NaCl solution a minute amount of active sodium phosphate was added. The blood was then defibrinated and allowed to circulate through an isolated liver for 2.5 hr. The labelled inorganic P present in the blood was determined at the start and at the end of the experiment and also the labelled phosphatide P of blood and liver at the end of the experiment. The ratio of the specific activity (activity per mgm P) of the blood phosphatide P to that of the blood inorganic P is seen from Table 1. In interpreting the figures of the table we should recall that if all phosphatides molecules present are newly formed the specific activities of the inorganic P and phosphatide P should be equal. The

TABLE 1

Specific activity of blood phosphatide P / Specific activity of blood inorganic P

	1	2	3 (average value)
Normal blood (av. of 3 exps.)	0.5×10^{-3}	1.2×10^{-3}	0.85×10^{-3}
Lipaemic blood (av. of 2 exps.)	1.6×10^{-3}	3.4×10^{-3}	2.5×10^{-3}

figure of 0.85×10^{-3} for the ratio quoted, for example, shows that the new formation of phosphatide molecules within the experiment amounts to only 0.085%. In the course of the experiment inactive inorganic P of the liver and also a part of the P present in the organic P compounds of the liver exchange with the active plasma inorganic P and lower the specific activity of the latter. The specific activities of the plasma inorganic P being thus different at the start and at the end of the experiment we have calculated the ratio (seen in Table 1) for the beginning of the experiment (col. 1), for the end (col. 2), and also an average value (col. 3).

The figures of Table 1 for normal blood hardly differ from the figures obtained in the experiments *in vitro* (average value 0.8×10^{-3}). While,

however, in the experiments *in vitro* no definite difference was found in the formation of phosphatides in normal and lipaemic bloods, in the perfusion experiment about three times as many newly formed phosphatide molecules were found to be present in the lipaemic blood as in the normal. This result is supported by figures obtained when investigating the labelled phosphatides extracted from the livers used in the perfusion experiments. Here also (see Table 2) a greater part of the phosphatide present became labelled when lipaemic blood was used.

Table 2

Liver	Phosphatide P per gm fresh tissue mgm	Total P per gm fresh tissue mgm	Spec. activity of phosphatide P × 100 / Spec. activity of inorganic P
	Perfusion with normal blood		
1	0.96	2.6	1.25
2	1.13	2.9	1.53
3	0.88	2.0	1.78
	Perfusion with lipaemic blood		
4	0.75	2.4	2.75
5	0.69	2.2	2.59
6	1.32	3.7	2.79

The livers used were taken from fasting cats, except in Exp. 3. In Exp. 6 the specific activities of the ester P of the liver and the protein P (remaining P after extraction with ether-alcohol and trichloroacetic acid) were determined as well. The relative figures obtained were; specific activity of the inorganic P, 1; of the ester P, 0.218; of the protein P, 0.068. The radioactive P atoms can only enter into the phosphatide molecules by a synthetic process. If the radioactivity of 1 mgm organic P of the liver were equal to that of 1 mgm inorganic P, all organic P atoms would have been replaced. If the organic P were not radioactive at all, none of the organic molecules could be newly formed. If all the phosphatide molecules present in the liver after the perfusion experiment had been newly formed the value of the ratio in the last column would be 1. From the figures it follows that 1.5% of all phosphatide molecules present in the experiment with normal blood and 2.7% in that with lipaemic blood are formed in the course of the experiment.

During the perfusion experiment some molecules may have decomposed thus introducing an uncertainty into all conclusions based on the determination of the amount of phosphatides present. Our conclusions

are not influenced, however, by this source of error, since they are not based on determinations of the phosphatide content before and after perfusion but on the ratio of labelled and non-labelled phosphatide molecules present in the liver. We may, therefore, conclude from the results described above that lipaemic blood is more effective in the formation of phosphatides in the liver than is normal blood. This result suggests that one of the main reasons for alimentary leicithinaemia is that during the influx of lipaemic blood phosphatide formation in the liver is increased and phosphatides are discharged into the circulation [cf. AYLWARD *et al.*, 1935]. As the lipaemic blood is changed into normal blood the excess phosphatides are taken up by the liver and other organs, until the "normal" phosphatide content of the blood is reached.

Summary

In experiments *in vitro* the amount of labelled phosphatide obtained in shaking blood with radioactive sodium phosphate is the same whether normal or lipaemic blood is used.

In perfusion experiments the lipaemic blood is found to contain more labelled and thus newly formed phosphatide than the normal blood. The same result applies also to the phosphatides extracted from the liver in the perfusion experiment.

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30. RATE OF PENETRATION OF PHOSPHATIDES THROUGH THE CAPILLARY WALL

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Ions or molecules of crystalline substances present in the plasma can easily penetrate through the capillary wall. As soon as a few minutes after injecting labelled sodium ions ($^{24}Na^{+}$) into the jugularis, we find these ions proportionally distributed between the sodium ($^{23}Na^{+}$) ions of the plasma and those of the interspaces. On the other hand, colloidal particles like those formed by the proteins of the plasma under physiological conditions pass through the walls of the capillaries at very slow rate only. The phosphatides present in the plasma can be expected to have an intermediary position as to their penetrability through the capillary wall between the crystalline constituents and the proteins present in the plasma. To determine the rate of penetration of the plasma phosphatides through the capillary wall, we introduced labelled phosphatides (phosphatides containing radioactive P) into the plasma and measured the rate of their disappearance from the circulation.

The labelled phosphatides were obtained in the following way. Labelled sodium phosphate was administered to a rabbit (A). The phosphatides formed, after the start of the experiment, in the liver and other organs of this rabbit become labelled; a part of these labelled phosphatides is liberated into the plasma. By injecting plasma of this rabbit (A) into the circulation of another rabbit (B), we introduced labelled plasma phosphatides under strictly physiological conditions into the circulation. To avoid the increase of the plasma volume of rabbit B, we removed, previous to the injection of the labelled plasma, for example, 20 cc. blood of rabbit B. This blood was, after addition of heparin, gently centrifuged to separate the bulk of its plasma content which was then replaced by the labelled plasma of rabbit A. The blood thus obtained was injected into the jugularis of rabbit B. This rabbit, thus, gets its own corpuscles reincorporated, combined with the corresponding amount of labelled plasma of the other rabbit. An aliquot part of the plasma of rabbit A is kept to be analysed.

The labelled phosphatide molecules introduced into the circulation of rabbit B become distributed in the total plasma of the rabbit almost

at once, the next step being the continuous escape of the labelled phosphatide molecules through the capillary wall and their replacement by other phosphatide molecules, originally located in the organs, which diffuse in the opposite direction, namely through the capillary wall, into the plasma. Since the phosphatide content of the plasma remains practically constant during the experiment, the exodus of a certain quantity of phosphatides must be followed by the influx of about the same amount. In view of the very minute turnover of phosphatides

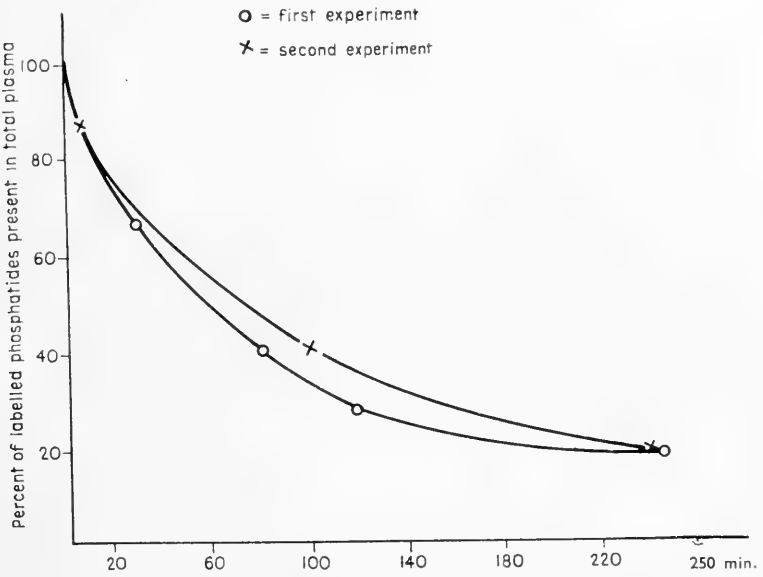

FIG. 1. Rate of disappearance of labelled phosphatide molecules from the plasma.

in the blood, the number of labelled phosphatide molecules which are decomposed in the plasma during the experiment can be neglected. The processes described above are going on under strictly physiological conditions. The replacement of ordinary phosphorus (^{31}P) by radioactive phosphorus (^{32}P) in some of the phosphatide molecules can certainly not be considered to entail the introduction of a non-physiological component into the circulation, as such a replacement cannot influence the chemical behaviour of the phosphatide molecules to any significant extent.

The rate at which the labelled phosphatides escape from the plasma of rabbits is seen in Tables 1 and 2, and also in Fig. 1. The figures of the tables were obtained by comparing the radioactivity of the phosphatides present in 1 cc. plasma samples of rabbit B, taken at different intervals, with that of the phosphatides of an equal plasma volume

of rabbit A. The phosphatides were extracted by making use of BLOOR's method. After being converted into phosphate by wet ashing, an aliquot part of the solution obtained was used in the colorimetric measurement of the P content, another to secure an ammonium magnesium phosphate precipitate, the activity of which was determined by a Geiger counter.

The calculation of the amount of labelled phosphatides present in the total plasma of the rabbit from that found in 1 cc. necessitates the knowledge of the total plasma volume. This was calculated from the blood volume and the known haematocrit value. The blood volume was determined by making use of a method recently described (HAHN and HEVESY, 1940). This method is based on the measurement of the dilution of a known volume of corpuscles containing labelled organic P compounds in the circulation of the animal, the blood volume of which is to be determined. In experiments on rabbits, the injection of foreign plasma was preceded by the removal of a corresponding volume of blood, as described above. In experiments on chicks, however, no blood was removed beforehand.

EXPERIMENTS ON RABBITS[1]

[1] Some of the results obtained were previously published by us in a note to *Nature* **144,** 204 (1939). — F. E. HAVEN and W. F. BALE [*J. Biol. Chem.* **129,** 23 (1939)] injected emulsions containing labelled phosphatides prepared from the liver of the rat into the circulation of another rat and found the labelled phosphatides to accumulate mainly in the liver and the spleen.

As seen in Tables 1 and 2 and also in Fig. 1, half of the labelled phosphatides introduced into the plasma leave the circulation by penetrating through the capillary wall in the course of about an hour. As the non-labelled phosphatides can be expected to show the same behaviour as the labelled ones, we can conclude that, from all phosphatide molecules present at the start of the experiment in the plasma, half will no longer be present after the lapse of about an hour, and will be replaced by others which were previously located in the organs.

TABLE 1. — RATE OF ESCAPE OF LABELLED PHOSPHATIDES THROUGH THE CAPILLARY WALL OF A RABBIT WEIGHING 2.4 kgm

First experiment

Time	Per cent of labelled phosphatides injected into the jugular vein, present in the total plasma
0 min.	100
30 „	65.8
82 „	39.8
120 „	27.2
247 „	17.6

Table 2. — Rate of Escape of Labelled Phosphatides through the Capillary Wall of a Rabbit Weighing 2.8 kgm

Second experiment

Time	Per cent of labelled phosphatides injected into the jugular vein, present in the total plasma
0 min.	100
7 „	85.6
100 „	40.1
242 „	18.4

Three objections may be raised against the conclusions drawn above: *a)* Labelled phosphatides can be decomposed in the plasma leading, for example, to the formation of labelled inorganic P; *b)* they can be incorporated into the corpuscles: *c)* they can be synthesised in the body of rabbit B, into which labelled plasma was injected. In that case, besides a loss of the labelled phosphatides introduced into the circulation of rabbit B, some gain of such phosphatides due to a synthesis of labelled phosphatides in rabbit B would take place.

The objections mentioned above are, however, not justified, as

a) We recovered (see Table 6) more than 1/2 of the labelled phosphatides injected into the plasma of rabbit B 4 hours later in the organs investigated, in spite of the fact that the latter did not include the skin, the skeleton, and large parts of the digestive tract, which presumably took up an appreciable part of the labelled phosphatides. Furthermore, in the course of 4 hours, a non-negligible part of the phosphatides present in some of the organs and, thus, also that of the labelled phosphatides taken up by these organs, was renewed. In the liver, about 1/6 of the phosphatides present was found to be renewed in the course of 4 hours[1]. In view of the above considerations, the amount of phosphatides decomposed in the plasma in the course of a few minutes can certainly be disregarded.

b) That in the course of a few hours the replacement of corpuscle phosphatides by plasma phosphatides is a restricted one, is seen from the following figures. In two experiments, after the lapse of 4 hours, 2 resp. 1.3 per cent of the labelled phosphatides originally present in the plasma of rabbits were found to be located in the corpuscles.

As to objection *c)*, the formation of labelled phosphatides does not take place in rabbit B to any significant extent in view of the absence of a sufficient amount of labelled phosphate. This fact is seen from the following consideration: We administered to rabbit A 5×10^6 counts as phosphate and found the next day in the plasma of this rabbit 40,000 counts. We injected into rabbit B 20 cc. plasma containing 8000 counts, of which 4000 were due to phosphatide P and 4000 to inorganic P. As from 5×10^6 inorganic P counts introduced 20,000 phosphatide counts were found after the lapse of a day in rabbit A, we can conclude that, within that time, less than 20 phosphatide counts were formed in rabbit B, thus an insignificant amount.

[1] G. Hevesy and L. Hahn, *Det Kgl. Danske Vidensk. Selskab, Biol. Medd.* **15**, 5 (1940).

EXPERIMENTS ON CHICKS

Labelled phosphate was administered by subcutaneous injection to chicks (A_1, A_2 and A_3, respectively). After the lapse of a day, plasma samples of these chicks were taken. One part (1 cc.) of the sample was injected into the jugularis of the chicks B_1, C_1, D_1, E_1, B_2, C_2, D_2 and B_3, C_3, D_3, E_3, F_3, respectively; another part was analysed. After the lapse of 7 to 67 minutes, plasma samples of chicks B, C, D, E and F, respectively, were taken and the activity of their phosphatide content determined; heparin was added to the blood before it was centrifuged. In Tables 3, 4 and 5, the results of these experiments are recorded. The time recorded in Tables 3 and 5 was reckoned from the middle of the time of injection, which took about one minute.

Table 3. — Percentage of Labelled Phosphatides Present in the Plasma of Chicks B_2, C_2, D_2, after Injection of 1 cc. Plasma of Chick A_2 Containing Labelled Phosphatides

Chick	Weight of the chick	Total plasma volume	Time	Per cent of the labelled phosphatide injected	
				present in 1 cc. plasma	present in the total plasma
B_2	114 gm	3.6 + 1 cc.	17.4 min	8.02	36.9
C_2	127 „	4.1 + 1 „	17.9 „	7.34	37.4
D_2	134 „	4.3 + 1 „	17.0 „	7.12	37.7

As seen in Table 3, after the lapse of 17.0 to 17.9 min, 1 cc. of the plasma of chicks B_2, C_2 and D_2, respectively, contains only about 7 per cent of the labelled phosphatide present in 1 cc. of the plasma of chick A injected into chicks B_2, C_2 and D_2, respectively. This decrease is partly due to a dilution of the labelled phosphatides present in 1 cc. by the non-labelled phosphatides present in about 4 cc. plasma of chicks B_2, C_2 and D_2, and partly to an escape of the labelled phosphatides through the capillary wall into the organs and its replacement by non-labelled ones previously present in the organs. As seen in the last column of Table 3, from 100 labelled phosphatide molecules introduced into the circulation of the chicks, only about 37 were present in the plasma after the lapse of about 17 min.

Since the labelled phosphatides cannot be expected to show a different behaviour from the non-labelled ones, we can conclude that 63 per cent of all individual phosphatide molecules originally present are no longer in the plasma of the chick after the lapse of 17 min., being replaced by phosphatide molecules originally located outside the capillary wall.

In the first experiment which we carried out on chicks (see Table 4), we have chosen another procedure. We compared the activity of 1 mgm phosphatide P extracted from 1 cc. plasma of chick A with the activity of 1 mgm phosphatide P extracted from 1 cc. plasma of chick B, C, D and E, respectively. Should the phosphatide concentration in the plasma of the different chicks used in this experiment be about the same, we could calculate from the data obtained the loss of labelled phosphatides through the capillary wall in the course of the first 7 and the consecutive 60 min. as well. When determining the phosphatide content of the plasma in our second experiment, we found, however, very pronounced differences between the plasma phosphatide contents of the chicks used. (Chick

$A_2 = 6.5$ mgm %; $B_2 = 7.5$ mgm %; $C_2 = 4.0$ mgm %; $D_2 = 4.8$ mgm %)[1]. From these variations in the phosphatide contents of the plasma we followed that from the data obtained in the first experiment, we cannot calculate the loss of labelled phosphatides by the plasma in the course of the first 7 min., while we can state the loss sustained in the interval between 7 and 67 min after the start of the experiment. It is this value which is recorded in Table 4.

Table 4. — Change in the Specific Activity of the Plasma Phosphatide of Chicks B_1, C_1, D_1, E_1, after the Injection of Plasma of Chick A_1 Containing Labelled Phosphatides

Chick	Weight of chick	Ratio of specific activity of the phosphatide P obtained after 7 and 67 min
B_1	138 gm	2.2
C_1	156 „	2.1
D_1	138[1] „	1.9
E_1	107[1] „	2.8

[1] These chicks have shown pronounced exudates due to E-avitaminosis and were kindly put at our disposal by Dr. H. Dam. The injection was kindly carried out by Mrs. Svendsen. The above figures do not permit us to draw any conclusion as to a difference in the permeability of, for example, the muscle capillaries of normal chicks and chicks suffering from E-avitaminosis. To arrive at such a conclusion it would be necessary to compare the labelled phosphatide content of the muscle tissue of normal chicks and of chicks suffering from E-avitaminosis at the end of the experiment.

EFFECT OF HISTAMIN

We also carried out experiments in which histamin was injected simultaneously with the plasma containing labelled phosphatides. The results of these experiments are seen in Table 5.

The administration of histamin did not much affect the appearance of chicks C_3 and D_3, while chicks E_3 and F_3 could not stand on their feet for the first 5—10 min. which elapsed after the injection of histamin. From the last mentioned two chicks, only small blood samples, about 0.4 cc., could be secured, while we collected several cc. from chicks which got no or only minor doses of histamin administered. The total plasma volume of the chick was calculated as described on p. 6. The average figure obtained for the labelled phosphatide content of one cc. plasma of chicks C_3, D_3, E_3, and F_3, to which histamin was administered. 20 min. after the start of the experiment is about 7. It is the same figure which was obtaines for the labelled phosphatide content of the plasma of chicks B_3 and B_2, C_2, D_2 (see Table 3) in one cc. No striking effect

[1] Comp. also the great variations in the phosphatide content of the blood of chicks found by F. W. Lorenz, J. L. Chaikoff and C. Entenman, *J. Biol. Chem.* **123,** 577 (1938).

of the administration of histamin on the permeability of the capillaries by phosphatides is, thus, found. In view of the large fluctuations shown by the values obtained in the experiments in which histamin was administered, the above result is, however, to be interpreted cautiously.

TABLE 5. — PERCENTAGE OF LABELLED PHOSPHATIDES PRESENT IN THE PLASMA OF CHICKS B_3, C_3, D_3, E_3, AND F_3 AFTER THE INJECTION OF 1 CC. PLASMA OF CHICK A_3 CONTAINING LABELLED PHOSPHATIDES

Chick	Weight of the chick	Time	mgm histamin-dihydrochloride per gm chick weight	Per cent of labelled phosphatides injected, present in	
				1 cc. plasma	total plasma per gm body weight
B_3[1]	113 gm	21 min.	0	8.1	37.8
C_3	104 gm.	23 min.	3×10^{-3}	5.5	23.8
D_3	95 ,,	23 ,,	5×10^{-3}	10.0	40.5
E_3	113 ,,	2 ,,	5×10^{-3}	4.0	18.5
F_3.....	124 ,,	19 ,,	1×10^{-2}	7	34.8

[1] Comp. also Table 3.

The permeability of different artificial membranes to phosphatides was investigated by SÜLLMANN and VERZÁR (1934). They found that through such membranes which are permeable to water blue and Congo red the plasma phosphatides can penetrate as well.

UPTAKE OF LABELLED PHOSPHATIDES BY THE ORGANS

In the preceding chapters, we discussed the rate at which labelled phosphatide molecules located in the plasma penetrate through the capillary wall. We will now describe experiments which were carried out in order to determine to what extent the various organs took up the labelled phosphatides which left the circulation. We arrive at these figures by extracting the phosphatides of the organs and by determining their activity.

In Table 6, the percentage of labelled phosphatides introduced into the circulation, present at the end of the experiment in several organs, is recorded in the third column. The fourth column of the table contains data on the labelled phosphatide content of the interspaces computed on the assumption that all the labelled phosphatides present are to be found in the extracellular volume. For the extracellular volume of the organs of the rabbit we utilised the figures arrived at by MANERY and HASTINGS (1939). In the fifth column, the distribution of the labelled

phosphatides between equal volumes of the plasma and the extracellular fluid of the organs in question is stated on the assumption that the labelled phosphatides are solely to be found in the interspaces of the organ in question. The conclusion to be drawn from the figures of this column are discussed on page 270.

TABLE 6. — LABELLED PHOSPHATIDES FOUND IN THE ORGANS OF RABBIT B AFTER THE LAPSE OF 4 HOURS

Organ	Weight	Percentage of the labelled phosphatides injected into the vein, present in		Distribution coefficient[1] of labelled phosphatides between equal volumes of extracellular water and plasma water
		the blood-free organ	1 cc. extracellular fluid[1]	
Liver	62 gm	28.9	2.17	9.8
Kidneys	9 „	0.88	0.19	0.85
Muscles	910 „	2.5	0.018	0.082
Heart	5 „	0.21	0.12	0.54
Spleen	1.2 „	0.06	0.16	0.72
Small intestine mucosa	46 „	1.1	0.065	0.29
Lungs	10 „	1.0	0.22	1.0
Brain	6 „	0.05	0.022	0.10
Plasma	79 gm	17.6	0.22	

[1] Calculated on the assumption that no penetration of labelled phosphatides into the cells took place

In another experiment, only the labelled phosphatide content of liver and muscles were determined. The liver, weighing 85 gm, contained 38 per cent of the labelled phosphatides injected after the lapse of 4 hours, while in the blood-free muscles, weighing 1060 gm, 2.7 per cent of the labelled phosphatides administered were present.

The figures given above relate to the labelled phosphatide content of organs of rabbits killed by bleeding. While such organs have only a comparatively small blood content, this cannot be entirely disregarded. Some of the labelled phosphatides present in the organs will be due to their blood content. In the muscles of the rabbit we found, by making use of the method of EICHELBERGER and HASTINGS (1937), that the blood content amounted to 0.5 per cent of the organs' weight. In the experiment described above, in which the weight of the muscles was 1060 gm and the total plasma of the rabbit amounted to 97 cc., the blood present in the muscles contained 1.0 per cent of the labelled phosphatides injected. In the case of the liver, the corresponding figure works out to be less than 1 per cent; in the case of the other organs the correction is insignificant.

As seen in Table 6, the labelled phosphatide content of all the organs but that of the liver can be interpreted as being present in the interspaces though this must not actually be the case. The liver contained, after the lapse of 4 hours, about ten times more phosphatides as can be explained by an uptake of the liver interspaces. This result suggests the explanation that not only the capillary wall but also the membrane of the liver cells is very easily permeable to phosphatides. The capillary wall of the small intestine, brain, and muscles is but fairly permeable, its permeability decreasing in the above sequence. The uptake of labelled phosphatides by 1 gm muscle makes out only about 1/170 part of the labelled phosphatides taken up by 1 gm liver. The corresponding figure for the small intestine mucosa is about 1/20.

FORMATION AND EXCHANGE OF PHOSPHATIDES IN THE LIVER

It is of interest to compare the amount of phosphatides synthesized in the liver with the amount which reaches the liver through an exchange process from the plasma. In the first case, we investigate the formation of labelled phosphatide molecules, in the second case no new labelled molecules were formed but all the labelled phosphatide molecules present were taken up by the liver from the plasma. This uptake is presumably followed by the release of a similar amount of phosphatide molecules previously present in the liver. An alternative explanation would be that the uptake of phosphatide molecules from the plasma by the liver is followed by a destruction of these molecules in the liver, the phosphatides lost by the plasma being replaced by phosphatides synthesised in other organs and liberated into the circulation.

As found by us, in the course of 4 hours 150 mgm liver phosphatides were newly formed, while during the same time 52 mgm phosphatides are carried from the plasma into the liver; if this amount is not replaced, at least to a large extent, by an equal amount of phosphatides migrating in the opposite direction, then it must be supplied by another source to the plasma. The organ responsible for such a supply must be one in which phosphatide molecules are formed at an appreciable rate. This is primarily the case—besides the liver—in the small intestine. We have, therefore, to ask it the amount of phosphatides supplied during 4 hours by the intestine into the circulation suffices to compensate the uptake of phosphatide molecules by the liver from the plasma. Süllmann and Wilbrandt (1934) determined the amount of phosphatides carried into the circulation by the intestinal lymph of the rabbit. They found that up to 1/2 mgm phosphatide P can be carried by the lymph stream in the course of 4 hours, thus appreciably less than given off by the plasma to the liver during the same time. As the amount of phospha-

tides brought into the circulation from the intestine does not suffice to compensate the loss of phosphatides by the plasma due to the uptake of phosphatide molecules by the liver, we can hardly expect the amount released by other organs to compensate the loss of the phosphatides. We have thus, to conclude that in the liver not only a very marked turnover of phosphatides takes place, but that phosphatide molecules exchange also with great ease between the liver cells and the plasma.

CALCULATION OF THE AMOUNT OF PHOSPHATIDES GIVEN OFF BY THE PLASMA TO THE LIVER

We saw that, after the lapse of 4 hours, 29 to 38 per cent of the labelled phosphatide molecules originally present in the plasma were found in the liver of rabbits. We wish to calculate from the average of these figures the total amount of phosphatides which, originating from the plasma, reached the liver in the course of 4 hours. When calculating this amount, we must envisage that large amounts of labelled phosphatides were taken up by the liver and, to some extent, by other organs as well and were replaced by non-labelled ones. These process clearly lead to an increase of the sensitivity of the radioactive indicator in the course of the experiment. While, at the start of the experiment, 1 count indicates, for example, 1 μ mgm phosphatide P, at the end of the experiment it will indicate the presence of 5 μ mgm.

Let us denote by L_0 the concentration of the labelled phosphatide molecules of the plasma at the start of the experiment, and by L_t that found after the lapse of t hours. The amount of phosphorus corresponding to L_0 (average of the values obtained in two experiments) was found to be 2.4 mgm. The decrease of the labelled phosphatide content of the plasma is assumed to take place according to the equation

$$L_t = L_0 e^{-\lambda t},$$

where λ is the constant of disappearance (analogous to the decay constant of radioactive bodies). If the liver alone would take up phosphatide molecules from the plasma, the amount of labelled phosphatides which, coming from the plasma, were located in the liver, would be equal to $L_0 - L_t$. As this is not the case, we must determine experimentally the percentage of the labelled plasma phosphatides present in the liver at the end of the experiment, which we denote by E. To arrive at the figure giving the percentage of the total amount of plasma phosphatide molecules (X) which were found in the liver after the lapse of t hours, we must multiply E by

$$\frac{\lambda t}{1 - e^{-\lambda t}} = Y .$$

From $\lambda = 0.69$ hour^{-1}, and $t = 4$ hours, it follows that

$$Y = 3 .$$

The value obtained for Y is too high, as the decrease of the labelled phosphatide content of the plasma takes place in the later stages of the experiment at a slower rate than according to the equation mentioned above. By taking into account this deviation we arrive at the values

$$Y = 2.6 \text{ and } X = 87 .$$

From the fact that the phosphatide content of the plasma of the rabbit amounted to 60 mgm it follows that, from the phosphatide molecules present in the liver after the lapse of 4 hours, 52 mgm were such as migrated from the plasma into the liver during the experiment.

Summary

Plasma of rabbits containing labelled phosphatides was injected to other rabbits. Plasma samples of the last mentioned rabbits were taken at intervals and their labelled phosphatide content determined. The labelled phosphatide content of the organs was determined as well. The labelled phosphatides were found to disappear at a fairly rapid rate from the circulation. Half of those originally present left the circulation in the course of about 2 hours.

The labelled phosphatide molecules penetrate at a fast rate into the interspaces of the liver, at a much slower rate into that of other organs; the sequence of the decreasing rate of penetration being lungs, kidneys, spleen, heart, small intestine, brain, and muscles.

The accumulation of labelled phosphatides in the liver in the course of 4 hours was ten times larger than expected in the case that the interspaces alone contained these phosphatides. From this fact follows a very great permeability of the cell walls of the liver to phosphatides. This is not the case for the other organs investigated. In view of the small amounts of phosphatides which penetrate, in the course of 4 hours, from the plasma into the muscles and the brain, we can conclude that the exchange of phosphatides between the cells of these organs and the circulation is almost negligible.

The total amount of phosphatides taken up from the plasma by the liver in the course of 4 hours was found to be 52 mgm. This uptake is accompanied by a migration of a similar amount in the opposite direction. Not only is the rate of turnover of phosphatides in the liver very high, the exchange of phosphatide molecules between the liver cells and the plasma takes place at a much higher rate than the corresponding process between other organs and the circulation.

In the course of 17 min, about 65 per cent of the labelled phosphatides originally present in the plasma of the chicks left the circulation.

Administration of large doses of histamin had no striking effect on the rate of penetration of phosphatides through the capillaries of the chick.

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31. ORIGIN OF PHOSPHORUS COMPOUNDS IN HENS' EGGS

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In this paper we discuss the origin of eggs phosphorus by making use of labelled (radioactive) phosphate as indicator. As the presence of labelled phosphorus in organic compounds proves that these compounds were synthetised since the administration of the labelled inorganic phosphate we can draw conclusions as to the place and time of formation of the lecithin and other compounds containing phosphorus and present in the egg, by making use of the above mentioned method. In the hope of finding which phosphorus compounds of the blood are responsible for the formation of the lecithin and possibly other phosphorus compounds of the egg we administered labelled sodium phosphate to hens by subcutaneous injection and investigated after some time the yolks removed from the ovary and further the composition of blood and of some of the organs. In other experiments eggs, laid at different times, were investigated. Finally we carried out also a few experiments *in vitro*.

Several of the compounds building up the egg contain phosphorus. Lecithin and other phosphatides form about one tenth of the yolk of the hens egg, the ratio of P to that of the other elements present in these compounds being about 1 : 25. From the phosphoprotein of the yolk vitellin is the most abundant, it contains on the average 0.54% P. The total of phosphoprotein P present in the yolk is somewhat less than half of the phosphatide P present, while only small amounts of nucleoprotein P are found, as seen in Table 1[1].

The phosphatide P content of the average yolk amounts to 60 mgm and its total P content to about 94 mgm. The total P content of the yolk is thus about 0.6% of its total fresh weight, while that of the white of the egg is much smaller, amounting to about 0.01%. The P content per gm of the small yolks found in the ovary is appreciably lower as seen from Table 2 and increases with the increasing size of the yolk.

[1] R. H. A. Plimmer and F. H. Scott, *Physiol.* **38,** 247, (1909.)

The phosphorus content of the shell of hens eggs is very variable, fluctuating between 0.1 and 0.3% of the shell weight. It may be of interest to recall that on the average 50% of the weight of the hens eggs is due to albumin, 39% to yolk and 11% to the shell.

TABLE 1. — PHOSPHORUS PRESENT IN THE YOLK IN PERCENT OF THE TOTAL PHOSPHORUS

Phosphatide P	61.4
Water soluble P	9.5
Phosphoprotein P	27.5
Nucleoprotein P	1.6

TABLE 2. — PHOSPHORUS CONTENT OF YOLKS

Weight of yolk in gm	Lecithin P in yolk in mgm	Total P in yolk in mgm	Lecithin P in 1 gm yolk	Total P in 1 gm yolk
0.03	0.03	0.049	1.00 mgm	1.63 mgm
0.1	0.20	0.26	2.00 ,,	2.67 ,,
0.694	1.72	3.57	2.49 ,,	5.18 ,,
2.51	6.25	13.0	2.49 ,,	5.2 ,,
4.63		4.00		8.7 ,,
7.68		93.75		12.2 ,,
13.6		125.0		9.2 ,,

[2] Lecithin plus other phosphatides.

According to general experience the yolk is formed while the growing egg is located in the ovary, about half of the white of the egg is formed by the albumin secreting portion of the oviduct, the shell membrane is deposited directly on this; and the more fluid portion of the albumin, constituting the second half of its entire bulk, enters through the shell membrane while the egg is in the isthmus and uterus. It has been found that the egg spends three hours in the glandular portion of the oviduct, one hour in the isthmus, sixteen to seventeen hours in the uterus including the time of laying.

PHOSPHORUS COMPOUNDS IN HENS BLOOD

The concentration of inorganic phosphorus, acid soluble phosphorus, lipoid (phosphatide) phosphorus and also of the total phosphorus present in blood, plasma and cells of chickens determined by HELLER, PAUL and THOMPSON[1] is shown in Fig. 1. The curves seen in the figure

[1] V. G. HELLER H. PAUL, and R. B. THOMPSEN, *T. Biol. Chem.* **106**, 357, (1934.)

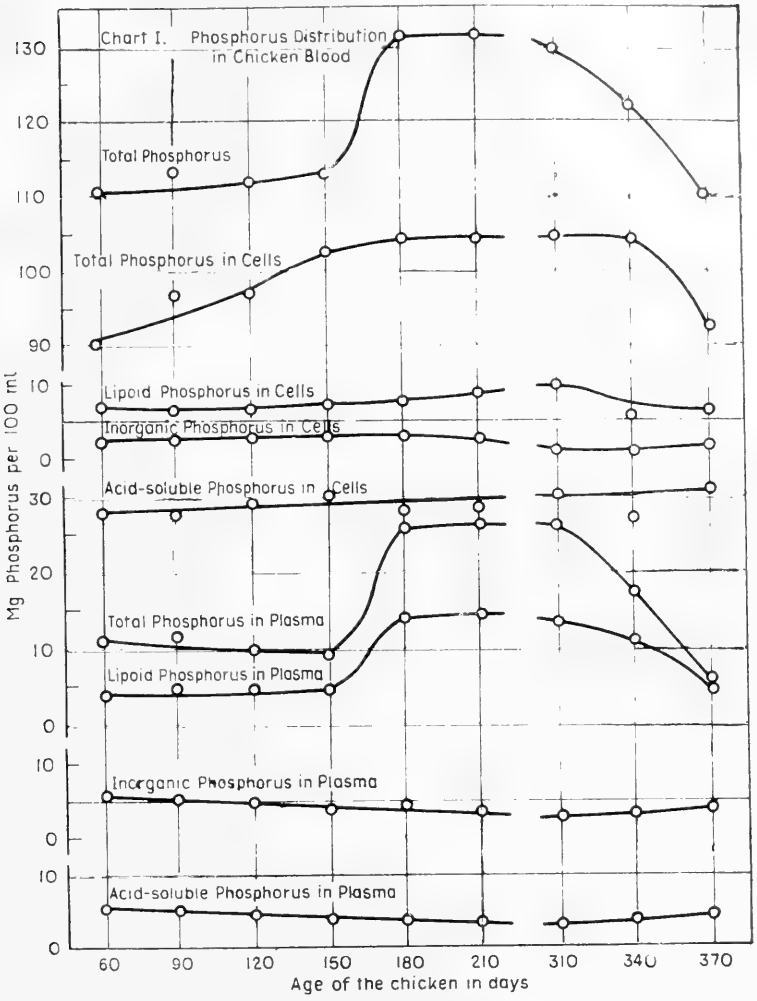

Fig. 1. Phosphorus distribution in chicken blood.

were obtained by analysing the blood of a large number of white Leghorn chickens. The analyses were repeated once a month or oftener beginning at the time when the chickens were 1 month old and continuing through the periods of growth, egg production, and subsequent molting. The results present very instructing data, they show that the phosphatide phosphorus alone, especially that of the plasma, changes very markedly with the age of the chicken, a rapid rise in the latter taking place after the lapse of 5 months at the time of production, this high level being held under the entire production period with some fluctuations and dropping quickly as production ceases and molting season approaches.

We determined the blood phosphorus of the laying hen denoted as I, the result being seen from Table 3. The blood phosphorus of another hen is discussed on p. 274.

Table 3. — P-content of Hens Blood

	mgm % in plasma	mgm % in corpuscles	mgm % in blood
Phosphatide P	20.0	22.6	20.7
Inorganic P	5.4	—	—
Total acid soluble P	—	53.1	21.3
Rest (Protein) P	9.4	31.8	16.8

In the blood of non-laying hens[1] after 24 hours fasting an average phosphatide P content of 16.8 mgm% was found the total plasma P amounting to 13 mgm%, the plasma inorganic P to 4.6 mgm%.

EXPERIMENTAL METHODS

The yolk was dried by adding ice cold aceton, the dry yolk was carefully pulverised and the powder obtained shaken for 15 min with 150 cc. ether, the last mentioned procedure being repeated four times using fresh ether. The ether was than carefully evaporated, the residue taken up with dry ether, the latter removed by evaporation, this time in a Kjeldahl flask, and the residue ashed.

The phosphatides of plasma, corpuscles and total blood were extracted by an ether-alcohol mixture after Bloor. The extract was several times carefully evaporated to dryness and taken up with ether or petrol ether. The residue of the first extraction was treated with trichloracetic acid (10 cc. of 10% solution for each cc. of blood) and from the filtrate obtained the inorganic P precipitated as ammonium magnesium phosphate; the esters present in the filtrate were hydrolised and the phosphate produced by the hydrolysis of the esters precipitated as ammonium magnesium phosphate. Though the extraction and the neutralisation of the acid solution were both carried out at −9°, some of the inorganic phosphorus present may be due to decomposition of the esters and we therefore gave in the table only the total acid soluble phosphorus present in the corpuscles which includes both the inorganic and the ester phosphorus.

The liver was minced, dried in vacuo at room temperature, pulverised, dried again in vacuo and extracted with ether-alcohol (1:3), the latter being left to boil for 15 sec. In one case we extracted with ether alone to compare the active P content of the ether soluble phosphatides such as lecithin with that of the total phosphatides. The acid soluble P was extracted from the dried liver powder by treatment with cold (−10° to −15°) solution of trichloracetic acid, first with a 10% solution for 10 min and than twice with a 5% solution each for 5 min. The inorganic and organic constituents of the acid soluble phosphorus were separated as stated above. The P content and activity of the residue obtained after extraction of the phosphatides and the acid soluble P was also investigated.

[1] H. M. Dyer and I. H. Roe, *J. Nutrit.* **7**, 623 (1934).

We determined the phosphorus content of a known fraction of the inorganic phosphate solution obtained in the above described procedures by the colorimetric method of FISKE and SUBAROW. The phosphate content of another fraction of the phosphate solution was precipitated in the form of ammonium magnesium phosphate and its activity determined by making use of a Geiger tube counter. Let us say we have administered a hen a labelled phosphate solution containing 1 mgm P and showing an activity of 10^6 counts per minute. We want to know what percentage of this labelled phosphorus will be found in the yolk lecithin. To arrive at this figure we take from our solution containing the labelled phosphorus as much as corresponds to $^1/_{10000}$ of the amount administered to the hen and precipitate the phosphate, denoting the precipitate obtained as our standard preparation, while we will call the precipitate obtained from the yolk lecithin as lecithin preparation. Before precipitating both the standard and the lecithin preparation we add to the solution a known amount, usually about 80 mgm, of inactive sodium phosphate, by so doing we diminish the amount of labelled phosphate possibly remaining in solution after precipitating with the magnesium citrate reagents and furthermore we obtain a standard and a lecithin preparation of equal weight. The β-rays emitted by the active phosphorus being to an equal extent absorbed in the two preparations the activity of which is to be compared, their weight and thus the thickness of the layers investigated being the same, there is no need to pay attention to the absorbtion of the β-rays in the samples investigated. Nor need the decay of the radioactive P be considered, as both the preparations to be compared, the lecithin and the standard preparation, decay at the same rate. The yolk residue obtained after removal of the lecithin was treated in similar way and also the white of the egg, while the shell was ignited and dissolved after ignition in hydrochloric acid, the solution being treated in the way described above. The samples were placed in small aluminium dishes having a surface area of 1.1 cm^2 and were placed immediately below the aluminium window of the Geiger-counter used.

Before discussing the results obtained we recall some facts about the circulation of labelled phosphorus in the blood.

Sensitivity of labelling

Let us start from labelled sodium phosphate preparation of such activity that when the later was first put into the blood, 1 mgm P will show 10000 activity units. As a result of a rapid exchange going on chiefly between bone phosphate and the inorganic phosphate of the blood 1 mgm will soon correspond to less than 10000 activity units. The total inorganic phosphate content of the blood remains constant, except in the case which we will not consider at present where a comparatively large amount is injected, while the individual phosphate ions will very soon be replaced to a large extent by other phosphate ions which were hitherto located in the skeleton or in other organs. After some time we shall find a large part of the labelled phosphate in the organs and the probability that the labelled phosphate leaves the organs and gets back again into the blood will increase, the effect of this re-entrance into the blood will be that with increasing time the net rate of decrease of the inorganic labelled phosphate content of the blood will be less and less. Loss of phosphate by excretion and by the formation of organic phosphorus compounds in the blood and in the organs will further complicate the curve representing the labelled P content of the blood as a function of time. We determined the latter experimentally for the blood of different animals and also of human subjects, but not for the hen, (Compare, however, the results given on page 281). The conclusions drawn in this paper do not necessitate

the knowledge of the change of the labelled phosphate content of the hens blood with time, it is for our present purpose sufficient to bear in mind that an initial rapid decrease of the labelled inorganic P content of the plasma occur and becomes slower.

In the first experiments described in this paper, in contrast to most of our experiments, we administered large amounts of P, of the order of magnitude of 100 mgm. The very strongly active phosphorus preparation (of a strength of about 10^6 counts) used in these experiments was a generous gift of Professor LAWRENCE and was prepared by the bombardment of few grams of red phosphorus by high speed deuterium ions generated in Professor LAWRENCE's powerful cyclotron. The active P was thus mixed with a comparatively large amount of inactive phosphorus. In the experiments to be described, in contrast to some other experiments, the comparatively large amounts of phosphorus did not interfere, their presence in the active preparation has even the advantage that we can fix exactly the limit within which the sensitivity of our indicator, the number of mgm of total inorganic P indicated by 1 count activity, varied throughout the experiment. The 100 mgm P administered had an activity of 10^6 counts. If the labelled P had not been diluted by non-labelled P of the organs we should have found after the lapse of 28 hours, the time of the experiment discussed on page 276, a specific activity of the plasma blood inorg. P—activity per mgm P—amounting to about 1% of the total activity administered. (The amount of inorg. P present in the total plasma is only about 5 mgm and thus much smaller than the 100 mgm P administered.) As seen from Table 9, however, only 0.01% was found, showing that from the inorganic P atoms present in the blood of the hen after the lapse of 28 hours only 1% were those actually administered, the rest being ones originating from different organs and partly also from the food taken within that time.

We carried out three types of experiments:

a) Administration of labelled sodium phosphate to a hen and investigation of the eggs layed at different dates.

b) Administration of labelled sodium phosphate, killing the animal, removal of the yolks and investigation of these yolks, the blood, the liver and other organs.

c) Experiments *in vitro* in which eggs were placed in labelled sodium phosphate solutions for few days and investigated as to what extent the labelled P penetrated into the egg.

We will first discuss experiments of the type *a)*.

a) Investigation of the labelled phosphorus content of eggs laid at different dates

We injected radioactive phosphorus as sodium phosphate subcutaneously to hens and investigated the radioactive phosphorus content of the different parts of the eggs laid at different times. The first egg was layed 4½ hours after administering the radicactive (labelled) phosphorus. We found the albumin to contain 0.0015% of the 40 mgm of phosphorus injected, a similar amount 0.0014% being present in the yolk. As the total phosphorus content of the yolk was found to be 100 mgm and that of the albumin only 4 mgm, the specific activity (active phosphorus per mgm normal phosphorus) was twenty-five times larger on the albumin than in the yolk. We found the lecithin phosphorus to be 53% of that of the total phosphorus of the yolk and to be entirely inactive. No synthesis of lecithin molecules took place in the yolk therefore within the 4½ hours preceding the laying of the egg, as in that case some active lecithin molecules should have been formed: taking this fact into account the specific activity of the other than lecithin phosphorus present in the yolk works out to be thirteen times smaller than that of

the albumin P. As 40 mgm active phosphorus were injected and only 0.0006 mgm are found in the albumen we can conclude that the formation of albumen from inorganic blood phosphorus in the course of the last 4½ hours which the egg spent in the oviduct is a very moderate one, even when we take into account that the 0.0006 mgm active phosphorus found in the yolk passed through the albumen into the yolk bringing the amount of labelled phosphorus present at least temporarily in the albumen to 0.0012 mgm and that a large part of the active phosphorus injected gets rapidly replaced in the blood by non-active phosphorus present in the skeleton and other organs.

In the shell of the egg we find 10 mgm phosphorus by chemical analysis (colorimetric method of FISKE and SUBBAROW) and 0.1 mgm of the labelled phosphorus administered by radioactive determination (measurements with a Geiger-counter). 1% of the shell phosphorus originates thus from the labelled phosphorus administered, which got into the shell in the course of the last 4½ hours before laying the egg.

The labelled phosphorus content of eggs layed at different time is shown by the figures of the tables 4 to 6.

In what follows we discuss the significance of these figures. That the specific activity of the shell is very much higher after 0.17 days than at a latter date is due to the fact that shortly after the administration of the labelled P the activity of the inorganic P of the plasma is very high and it is the latter which is incorporated into the shell. As found by us in numerous cases the active P content of the plasma decreases first rapidly and later at a decreasing rate the difference between the specific activity of the plasma and that of the tissues becoming less and less. The specific activity of the shell phosphorus is a measure of that of the inorganic plasma P at the time of formation of the shell and vice versa. The low specific activity of the albumin P in the egg layed after 0.17 days comes possibly for the following reasons. The white of the egg was already to an appreciable extent formed before the administration of the labelled P. The phosphorus compound of the plasma, presumably the plasma protein which mainly enters into the white was at such an early date after the administration of the labelled P active only to a small extent. The synthesis of labelled organic compounds takes some time and therefore shortly after the administration of labelled P the specific activity of the inorganic plasma P is much higher than that of the organic P. On the other hand the labelled organic P disappears at a slower, usually even much slower, rate from the plasma than the labelled inorganic P, the latter having a unique opportunity to exchange with the inactive tissue, especially bone tissue P.

When comparing the yolk figures with those of the albumin we have to bear in mind that contrary to the albumin which is formed within the day preceding the laying of the egg the greater part of the yolk was already present when the active phosphorus was injected and therefore the labelled phosphorus of the yolk was diluted by the unlabelled phosphorus already present in the yolk. With increasing time we should expect the amount of active phosphorus in the yolk to increase.

Labelled P administrated at different dates

In another set of experiments we were interested in producing strongly active egg-lecithin to find out whether after feeding the latter as dry yolk to rats, the presence of active lecithin in the blood of the rats can be ascertained. This was found not to be the case. In these experiments we administered to the hen on several days active phosphorus which made the interpretation of the activity-measurement of the yolk removed from the ovary rather difficult. A comparison of the activity of the shell of the yolk with its fluid interior revealed large diffe-

rences. The semi-solid yolk shell formed from very active blood was found in one case to be seven times more active than the fluid interior of the yolk, and five times in another case. With decreasing size of the yolk the difference between the specific activity of the yolk phosphorus originating from the inner and the outer part of the yolk diminished and finally vanished.

TABLE 4. — ACTIVE PHOSPHORUS CONTENT OF EGGS. HEN I.

Egg laid after administration of active phosphorus	Percentage of active phosphorus administered found in: Shell	Albumin	Total yolk	Yolk lecithin
0.17 days	0.24	0.0015	0.0014	0.000
1.0 ,, ...	0.052	0.032	0.109	0.014
3.0 ,, ...	0.036	0.030	0.42	0.17
4.5 ,, ...	0.026	0.027	0.95	0.34
6.5 ,, ...	0.022	0.020	0.85	0.35

TABLE 5. — HEN I.

Egg laid after administration of active phosphorus	Percentage active phosphorus administered found in 1 mgm egg phosphorus × 10^3. (Specific activity × 10^3). Shell	Albumin	Yolk after removal of phosphatides	Yolk phosphatides
0.17 days	24.0	0.38	0.03	0
1.0 ,, ...	5.2	8.0	2.0	0.26
3.0 ,, ...	3.6	7.5	5.1	3.3
4.5 ,, ...	2.6	6.8	12.6	6.4
6.5 ,, ...	2.2	5.0	10.4	7.0

TABLE 6. — DISTRIBUTION OF THE ACTIVE PHOSPHORUS ADMINISTERED BETWEEN DIFFERENT PARTS OF THE EGG

Egg laid after administration of active phosphorus	Shell %	Albumin %	Yolk after extraction of phosphatides %	Yolk lecithin phosphatides %
Hen I.				
0.17 days	98.9	0.6	0.5	0.0
1.0 ,, ...	27.0	17.0	48.8	7.2
3.0 ,, ...	7.0	6.0	50.9	36.1
4.5 ,, ...	2.6	2.7	60.5	34.2
6.5 ,, ...	2.5	2.2	56.0	39.3
Hen II.				
0.5 days	46.1	35.0	18.9	
3 ,, ...	30.5	6.8	38.2	24.5
4 ,, ...	15.0	6.2	56.4	32.4
6 ,, ...	7.2	2.8	58.5	31.5

b) Specific activity of yolk phosphorus

We administered to a hen 100 mgm. P as sodium-phosphate showing an activity of 10^6 counts and killed the hen after the lapse of 28 hours. From the ovary 34 yolks were removed and from the oviduct one egg. The weights of these are recorded in Table 7.

Table 7

Weight of yolk	Specimens present
About 30 mgm	20
„ 100 „	9
„ 700 „	1
„ 2500 „	1
„ 5000 „	1
„ 7500 „	1
„ 13000 „	1
(Egg) 18000 „	1

The specimens of 700 mgm and more were treated separately while averages of the 30 mgm and the 100 mgm. yolks were taken. The lecithin was extracted by ether and the residue brought into solution as described above. The results obtained are seen in Table 8.

The specific activity of the total P shows a maximum in the case of the 2500 mgm yolk. This result, puzzling at first sight, can be easily understood after consi-

Table 9. — Specific Activity of Yolk Phosphorus

(Percentage of the activity administered present in 1 mgm P)

Weight of yolk	Phosphatide P %	Non Phosphatide P %	Total P
30—100 mgm	0.00055	0.018	0.0073
700 „	0.00814	0.0173	0.0129
2500 „	0.0147	0.0186	0.0164
4600 „	—	—	0.0090
7700 „	—	—	0.0055
13600 „	—	—	0.0044

dering Fig. 2 taken from a paper of H. Gerhartz[1], in which the daily increase in weight of the yolks of a hen is recorded. The yolk grown from active blood and thus active will be diluted by the non-active yolk already present and this dilution will lead to a decrease of the specific activity of its P content. The dilution being least in the case of the 2500 mgm yolk, (comp. Fig. 2) its specific activity is bound to be highest. It takes some time after administration of the labelled sodium phosphate until labelled lecithin is transported into the plasma whereas

[1] H. Gerhartz, *Arch. dtsch. Ges. Physiol.* **156**, 215 (1914).

inorganic P of very high activity is present almost at once after injecting the active sodium phosphate. The non phosphatide P of the yolk is partly inorganic P which gets into the yolk in the early stage of the experiment when its specific activity is very high; we must therefore consider the lecithin P and not the non-lecithin or total P content as a proper measure of the growth of the yolk. From the fact that the lecithin P of the 30—100 mgm yolks became active only to a very slight degree we must conclude for example that they had hardly grown within the last 28 hours. When discussing the labelled lecithin P (phosphatide P) present in blood and in some of the organs we shall find definite evidence that the yolk lecithin is drawn from the plasma lecithin.

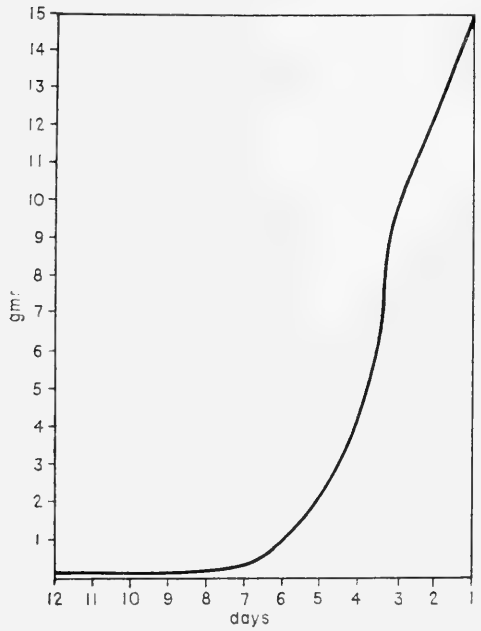

Fig. 2. Increment of the weight of yolks in the course of 12 days before completion of the yolk according to Gerhartz.

It is of interest to remark that the ratio of the total active lecithin content of small yolks, such as would require 10 days or more to attain completion, is a quantitative measure of their relative growth since the administration of the labelled P. When, however, comparing the lecithin P activity of a small yolk which increases its weight in the course of a day only to a slight extent with that of a large yolk growing at a rate of few gm per day the ratio of the total activities will not always be a correct measure of the growth since the administration of the labelled P. It may happen (comp. Fig. 2) that the growth of yolk per hour is larger at the end than in the beginning of the experiment the latter process determining thus to a larger extent the total growth within the time of experiment. From which it follows, that if at the beginning of the experiment the specific activity of blood lecithin happens to be greater than at the end, we underestimate the growth of the large yolk.

It is, however, the determination of the slow rate of growth of the small and tiny yolks, often present in a very large number in the ovary, which can be of special interest and which can hardly be determined by any method other than that outlined above.

Investigation of blood phosphorus

Plasma and corpuscles of the hens blood were separately investigated using the experimental method described on page 276. The results obtained are seen from Table 9 which contains data on the specific activity (activity per mgm P in percent of that injected) and also the total phosphorus present in the hens blood under

the assumption that the volume of blood of the hen amounted to 150 cc. and the volume of the blood plasma to 100 cc.

TABLE 9. — SPECIFIC ACTIVITY AND TOTAL PHOSPHORUS CONTENT OF THE HEN'S BLOOD

Fraction	Specific activity	Total phosphorus content
Plasma inorganic	0.0104	5.4
Plasma phosphatide	0.0125	20.0
Corpuscles phosphatide	0.0046	11.3
Corpuscles acid soluble	0.0036	26.5
Corpuscles protein	0.0031	15.9

That the specific activity of the plasma phosphatide P is greater after 28 hours than that of the inorganic P is due, as discussed on page 279, to the rapid disappearance of the individual inorganic P atoms from the plasma. In the experiment discussed on page 286, in which the hen was killed only 5 hours after the administration of the labelled P, the specific activity of the phosphatide P was found to be only 42% of that of the inorganic P.

It is of great interest that the specific activity of the plasma phosphatide P is several times larger than that of the corpuscle phosphatide which shows that a much smaller percentage of the corpuscle phosphatide than of the plasma phosphatide is renewed in the course of the experiment. This is an interesting result as it definitely disposes of the often discussed possibility that the blood phosphatide is synthetised in the corpuscles. Some of the corpuscles being formed during the experiment from labelled plasma are bound to contain labelled phosphatides; labelled phosphatides can furthermore easily get into the stroma of the corpuscles which are partly composed of phosphatides.

A very suggesting change in the phosphatide content of hens blood at the time of production was ascertained by HELLER, PAUL, and THOMPSON (comp. Fig. 1). The most interesting feature of the curves recorded by them is a gradual increase in the total P of the blood at the time of production, this high level being held during the entire production period with some fluctuations and dropping quickly as production ceases and molting season approaches. The increase is due to that of the lipoid P and is much more conspicious in the case of the plasma than in that of the corpuscles; the lipoid P content of the plasma is higher all through than that of the corpuscles, at the peak of production the former value being nearly three times higher than the last mentioned one. As about $^2/_3$ of the blood volume is composed of plasma it follows, that from the total lipoid P present in the blood $^5/_6$ are to be found in the plasma. The predominance of phosphatide P in the plasma found for laying hens is entirely unique as seen from the figures of Table 10, but understandable if we envisage the great strain put on the organism of a hen as to lecithin supply. A hen laying daily has to produce about 60 mgm lecithin[1] P a day; taking a total plasma volume amounting to 100. cc the total lecithin P of the plasma works out to be 20 mgm. If the lecithin found in the yolk is, as suggested from our results, taken from the plasma lecithin then the plasma has to give off three times its total lecithin content in the course of a day thus

[1] Lecithin plus other phosphatides.

putting an appreciable strain on the lecithin circulation. A strain which would be still more pronounced in the case of a lower plasma lecithin content.

Table 10. — Phosphatide P in Plasma and Cells of Different Animals

	mgm % P in		
	Plasma	Cell	Ratio $\frac{\text{cell}}{\text{plasma}}$
Rat	2.6	10	3.8
Rabbit	3.3	12	3.6
Man	9	19	2.1
Dog	14	14	1
Laying hen	14—20	8—23	0.87

Protein phosphorus in the hen's blood

After removal of the phosphatides and the acid soluble phosphorus, the remaining P is generally assumed to be present as protein P. The protein P content 31.8 mgm% found in the corpuscles of the hen in the 28 hours experiment is much higher than in the corpuscles of the blood of other animals, the corpuscles of the rabbit containing for example, as found by Mr. Aten, 4.4 mgm %. The same considerations apply to the protein P content of the plasma, which was found to amount to 9.4 mgm% for the blood of the hen in question and of 7 mgm% in the case of the hen discussed on page 276 while the plasma of a rabbit, for example, was found to contain only 0.03 mgm % protein P. From the high value of the specific activity of the protein P in the 5 hours experiment it follows that the protein phosphorus compounds present in the plasma were renewed even at a higher rate than those of the phosphatides. This result suggests a great participation of the plasma phosphorus protein in the formation of the egg. To arrive at a final conclusion as to the relation between the phosphorus protein compounds of the plasma and those of the yolk and white is difficult because of the fact that we lack simple methods of separation of the protein compounds. Vitellin, for example, can only be isolated by a very tedious and lengthy process and the isolation and separation of the blood protein phosphorus compounds are still more difficult, partly because only small quantities of these substances can be secured in the experiment. The fact that we have to base our conclusions on the amount of phosphorus present in the residue, remaining after extraction of the phosphatides and the acid soluble phosphorus compounds makes the result obtained less trustworthy than those arrived at when investigating the phosphatides, for example. The high value for the protein phosphorus of the corpuscles found by us, which may to some extent be due to an incomplete separation ot the phosphatides and acid soluble P, is supported by the data obtained by Heller, Paul and Thompson. They find for the total P present in the cells of laying hens about 100 mgm %, but only about 40 mgm % for the sum of inorganic acid soluble and lecithin P. The discrepancy suggests the presence of a further not investigated P fraction, which might be protein P. In the case of the plasma phosphorus the curves of Heller and his colleagues show the anomaly mentioned only to a smaller degree; the total phosphorus found by them is not very much larger than the sum of the acid soluble and phosphatide P.

The high protein P content of the blood plasma of a laying hen has presumably the same biological significance as the high phosphatide P content, namely a reduction of the strain put on the protein resp. phosphatide producing and carrying system in the organism of laying hens.

Phosphatide content of the liver

We extracted the total phosphatide content of the liver of a hen 28 hours after the administration of the labelled P, using the method described on page 277. Since we were interested in seeing whether lecithin soluble in ether shows the same specific activity as the total phosphatides we extracted another part of the liver tissue with ether alone. We found no marked difference, as seen from Tables 11 and 11a, which also contain data on the specific activity of inorganic and acid soluble (other than inorganic) P of the liver.

As seen from Table 11 the specific ativity of the liver phosphatide P is 56% of that of the inorganic P, from which it follows that about one half of the phosphatide molecules are labelled and thus formed after administration of the labelled

Table 11. — Specific Activity of the Liver P
(Activity per mgm. P)

Fraction	Specific activity
Total phosphatides (ether-alcohol extract) .	0.0152
Lecithin (ether extract)	0.0158
Inorganic	0.0272
Acid soluble, other than inorganic	0.0224

sodium phosphate. This result must, however, be interpreted with great caution. As already mentioned on page 277 in the early stage of the experiment the specific activity of P of the plasma is much higher than in the latter stages and the inorganic P of the liver was also more active at the early stage. This change of the specific activity with time would not affect our results if the specific activity of the phosphatide P should decrease with time at the same rate as does that of the inorganic P. That is, however, not the case. The phosphatide molecules can mainly escape from the circulation at an appreciable rate into the yolk, while the individual inorganic P atoms present in the plasma can rapidly exchange with such present, for example, in the skeleton, the latter being a much faster process in view of the huge extent of the skeleton. Therefore, when drawing conclusion

Table 11a — Percentage of Labelled P Administered Found in Plasma, Corpuscles and Liver

Fraction	Total Plasma (100 gm) %	Total Corpuscles (50 gm) %	Total Liver (41 gm) %
Phosphatide P	0.25	0.052	0.608
Inorganic P	0.056	—	1.643
Total acid soluble P	—	0.100	
Protein P	0.176	0.050	
Total P	0.482	0.202	2.251

from the comparison of the specific activities of the phosphatide P and the inorganic P as to what extent the phosphatide molecules got labelled we are apt to get values which are possibly too high. A trustworthy value could be obtained by keeping the specific activity of the inorganic P of the plasma constant by continuous injection of labelled phosphate of varying concentration and by thus avoiding a decrease in the specific activity of the inorganic P of the plasma, which is used for the synthesis of the phosphatide molecules in the liver and elsewhere. In the above case we can, however, conclude that a very appreciable part of the liver phosphatide molecules most have been renewed within the 28 hours of the experiment. In experiments on isolated livers in which the skeleton and other organs are not present it is easy to calculate from the ratio of the specific activities of inorganic P and phosphatide P the amount of newly formed phosphatides. In an isolated liver of a cat in the course of 2.5 hours about 1% of the phosphatide molecules present are newly formed. If in the course of 2.5 hours in an isolated liver of a cat about 1% of the phosphatide content is renewed there can be hardly any doubt that in the liver of a living hen in the course of 28 hours a large part of the phosphatide found is synthetised during that interval; in the liver of a living animal the enzymatic and other actions necessary for the synthesis of phosphatides are certainly as abundant as in an isolated liver and the phosphatide formation in the liver of a laying hen could hardly be less than in that of a cat. We are led to the same conclusion by the following consideration. The daily amount of phosphatide P tranfered from the plasma into the ovary is, in the case of the hen in question, which was laying one egg every other day, about 50 mgm. The main source of phosphatide production is, as we will see, the liver, and an amount not very far from 60 mgm must therefore have been produced daily in the liver of the hen. Since the latter containing 38 mgm of phosphatide P, a large amount of the liver phosphatide must have been renewed during the experiment. A similar conclusion applies to the plasma phosphatides, the 50 mgm phosphatide P being carried by the plasma, the total content of which is 20 mgm, the plasma phosphatide molecules must have been renewed repeatedly.

We are thus led to the result that the main source of phosphatides in a laying hen is the liver and that more than one half of the phosphatide molecules present in the hens liver were newly formed during the 28 hours preceding the administration of labelled phosphate. That the greatest part of the phosphatides is formed in the liver of a laying hen and reach the ovary through the plasma is very clearly shown in an experiment in which the hen was killed only 5 hours after administration of the labelled phosphate.

The acid soluble P of the liver, other than inorganic, mainly derived from P ester, shows, as seen in Table 11, a higher specific activity than the phosphatide P present in the liver.

Experiment on a hen killed after five hours

3.8 cc. of physiological sodium chloride solution containing 10 mgm labelled sodium phosphate were injected subcutaneously into a hen which weighed 1800 gms. The hen, which layed previously one egg daily weighing about 45 gm was killed after the lapse of 5 hours. The results obtained are seen in Table 12. Two separate determinations were carried out, the values found and also their average are given.

As seen from Table 12 the specific activity of the phosphatide P, which is a measure of newly formed phosphatides, is by far the greatest in the liver and markedly higher than that of the plasma phosphatide P. Contrary to the 28 hour

experiment, where the percentage of newly formed phosphatide molecules in the plasma nearly reached that found in the liver, in the 5 hours experiment the concentration gradient in the flow of labelled phosphatides directed from the liver into the plasma is very clearly shown (comp. Fig. 3). The percentage of labelled molecules in the ovary phosphatide is, on the other hand, much smaller than in the plasma phosphatides. From this it follows that the labelled phosphatide molecules present in the ovary were within 5 hours only partly replaced by ones present in the plasma. We investigated a yolk weighing 1.0 gm. The figures obtained are given in Table 12. A second yolk investigated weighed 2.7 gm. and its lecithin P had a specific activity of 0.0050. The specific activity of the yolk lecithin of the first mentioned yolk was found to be about $^1/_{11}$ of that of the plasma lecithin. From these figures it follows that about $^1/_{11}$ of the 1.0 gm i. e., 0.09 gm of yolk were grown within 5 hours. The actual growth was, however, presumably greater than 0.09 gm, since in the early stages of the experiment the plasma phosphatide was only very slightly active and so was the yolk tissue formed in this phase of its development. The fact that

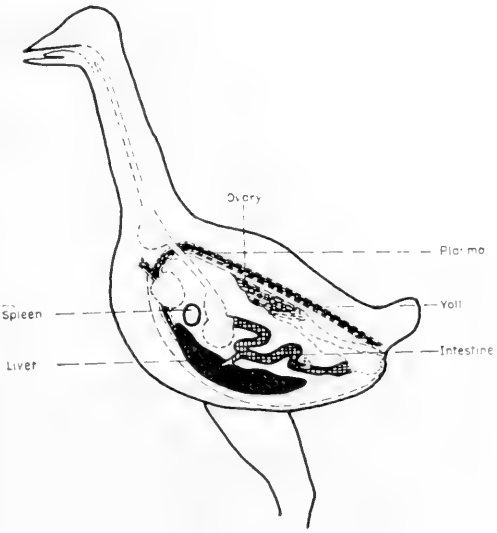

FIG. 3. The heaviness of the shading indicates the specific activity of the lecithin P and thus the percentage of the phosphatide molecules formed within the last five hours in the total phosphatides of the organ in question.

TABLE 12. — SPECIFIC ACTIVITY OF PHOSPHATIDE P

Organ	Specific activity (% of activity given, found in 1 mgm P)		Relative specific activity; that of the inorganic plasma P taken = 1
	Single values	Average	
Liver	0.094 0.082	0.088	0.54
Plasma	0.069 0.069	0.069	0.43
Ovary	0.0064 0.0064	0.0064	0.039
Yolk	0.0053 0.0075	0.0064	0.025
Intestine	0.018 0.018	0.018	0.11
Spleen	< 0.02	< 0.02	< 0.1

the specific activity of the ovary phosphatide was found to be low, as low as that of the yolk, proves definitely that the role of the ovary is not production of phosphatides but their extraction from the blood plasma together with other suitable constituents. The combination of phosphatides with proteins giving the characteristic composition and consistency of the yolk, is evidently one of its principal functions. In the experiment described above the specific activity of the P of the yolk soluble in trichloracetic acid was found to be 0.035, thus $^1/_{4\,5}$ part of that of the inorganic P of the plasma the latter being 0.16. Making the assumption that most of the acid soluble P originates from the inorganic P of the plasma we find a growth of the yolk amounting to $^1/_{4.5}$ part of its weight of 1.0 gm during the experiment. While the above mentioned figure of $^1/_{11}$ was, as already mentioned, a lower limit of the part of the yolk newly formed within 5 hours, the figure of $^1/_{4\cdot5}$ is a higher limit. A part of the acid soluble yolk phosphorus was formed at an earlier stage when the specific activity of the plasma inorganic P was appreciably higher than at the end of the experiment, and as our calculation is based on the specific activity of the plasma inorganic P at the end of the experiment it gives too high a value for the amount of yolk formed during the experiment.

The phosphorus of the white of the egg removed from the oviduct had a low specific activity, namely 0.0013. This is an interesting result in view of the strong activity shown by the phosphorus compounds present in the plasma (comp. Table 13). A possible explanation of this result is that some of the phosphorus present in the protein or other compounds of the oviduct tissue is utilised to produce the phosphorus compounds present in the white of the egg. In the course of five hours perhaps the compounds present in the tissue of the oviduct get labelled only to a slight extent. An other explanation is that while the average plasma protein P has a high specific activity 0.15 after the lapse of five hours, the specific activity of the phosphorus of one of the components of the protein mixture might be low. We are now engaged in the investigation of the origin of the phosphorus present in the white of the egg.

Table 13. — Specific Activity of Plasma Phosphorus

Fraction	Specific activity
Inorganic P	0.16
Lecithin P	0.069
Protein P	0.14

c) Experiments *in vitro*

We placed eggs in a neutral physiological sodiumphosphate solution containing 30 mgm P for 24 hours and investigated the activity of the different parts of the eggs, the results being seen in Tables 14 and 15.

The comparatively high labelled P content of the shell is due to phosphate exchange processes between the large shell surface and the solution and possible also to the formation slight amounts of calcium phosphate from the carbonate of the shell. An investigation of the activity of the lecithin extracted from the yolk gave an entirely negative result, this in agreement with the observation recorded on p. 279 that after the egg left the ovary no more lecithin formation takes place.

Table 14. — Ratio of the Specific Activity of Egg P and Solution P

	Shell	Albumin	Yolk
Egg I (Total P)	1.8×10^{-1}	1.4×10^{-3}	1.9×10^{-5}
Egg II (Total P)	2.0×10^{-1}	1.5×10^{-3}	4.0×10^{-5}

Table 15. — Distribution of the Active Phosphorus Taken up between the Different Parts of the Egg

	Shell %	Albumin %	Yolk %
Egg I	99.46	0.44	0.10
Egg II	99.40	0.41	0.19

DISCUSSION

We saw that by investigating the labelled phosphorus content of eggs or yolks we could draw conclusions as to the growth of the egg or yolk since the date of administration of the labelled P. It is, for example, possible to show that while the egg is in the oviduct not only is no more yolk formed but also no new lecithin molecules are synthetised. Should suitable enzymes be present, new and thus labelled lecithin molecules could be formed without any growth of the yolk. The ovary of a laying hen contains numerous tiny yolks growing at a slow rate; by comparing the incorporation of labelled P by such yolks, we get a quantitative measure of their relative growth since the administration of the labelled P. When comparing the growth of small yolks with large ones we can usually not obtain strictly quantitative results as to the relative growth because of the much more rapid relative growth of large yolks compared with that of small ones.

Placing eggs in a solution containing labelled P for some days we find the shell to contain an appreciable part of labelled P, while the amount shown by the white and especially by the yolk is very small, though easily measureable, even in the case of the yolk. No formation of labelled lecithin is, however, found in the yolk.

As to the formation of lecithin in the growing yolk, we arrive at the following result: The phosphatides found in the yolk are synthetised at least to a large extent in the liver and are transported through the plasma to the ovary which extracts the phosphatides. This is most clearly seen in the experiment in which the hen was killed only 5 hours after the administration of the labelled sodium phosphate. In this experiment the specific activity of the liver phosphatide P reached 54% of

that of the plasma inorganic P, while the specific activity of the plasma phosphatide P was appreciably smaller, amounting to only 43%; that of the ovary was very much smaller, namely 3.9%, and about as large as that of the strongest yolk phosphatide P. In the 28 hours experiment as to be expected, the difference in the specific activities was much smaller, the specific activity of the liver phosphatides being only somewhat higher than that of the plasma phosphatides. In the 28 hours experiment on the hen which used to lay one egg every other day the amount of phosphatides passing through the plasma on the way into the ovary was, in the course of the experiment, about twice the amount of phosphatides present in the plasma. In the 5 hours experiment, in which the hen experimented on was laying one egg daily, the amount, of phosphatide passing the plasma on the way into the ovary was about half the amount present in the plasma. From the low specific activity of the phosphatide P, that is from the low percentage of newly formed phosphatide in the ovary, it follows that in this organ only an insignificant amount of phosphatide can be formed. We have also to consider that a part of the labelled phosphatides found in the ovary is due to the presence of blood containing the latter. The specific activity of the plasma phosphatide P being appreciably smaller than that of the liver the labelled phosphatides must have come from the liver into the blood and not vice versa. By carrying out experiments *in vitro* with blood containing labelled sodium phosphate we found only a slight formation of labelled phosphatides, which is in accordance with the above conclusion.

The formation of phosphatides in the intestinal mucose by using radioactive phosphorus as indicator was first shown by Artom, Perrier, Santagello, Sarzana and Segré[1]. They found in an experiment, carried out on a rat, that after injecting labelled sodium phosphate the phosphatides extracted from the gut after a few days showed a specific activity only exceeded by that of the liver phosphatide P, the ratio of the specific activities being 1.2. The phosphatide production in a laying hen is larger than in any other animal of similar size, as the amount produced daily to be incorporated in the yolk is as much as about 2 times that present in the liver which contains more phosphatide than any other organ. The laying hen is, therefore, a very suitable animal for studying phosphatide formation. In our 5 hours experiment the specific activity of the intestinal phosphatide P is much smaller than that of the liver phosphatide P and also than the plasma phosphatide P. The bulk of the labelled P present in the plasma can, therefore, not originate from the intestinal phosphatide and the latter can not be the chief source of the yolk phosphatide. The phosphatides formed in the

[1] *Nature* **139,** 836 (1937).

intestine can, however, have and presumably actually do have a role in the supply of the plasma phosphatides. The presence of phosphatides in the intestinal lymph was repeatedly shown[1] in experiments on dogs. The amount of phosphatides reaching the hens circulation by the influx of intestinal lymph could be ascertained by measuring the amount of intestinal lymph produced and also its phosphatide content. In Fig. 3 we show the specific activities of the phosphatide P in the organs of the hen killed 5 hours after the administration of the labelled sodium phosphate. The heaviness of the shading indicates the specific activity.

A hen laying daily deposits about 60 mgm phosphatide P in the yolk or about 3 times as much phosphatide as present in the plasma. In the course of a day the phosphatide content of the plasma of a laying hen must therefore be replenished three times. In view of this great strain on the phosphatide circulation in the plasma it is very significant that the plasma phosphatide content of a laying hen is higher than in most other animals. If the laying hens plasma should show such a low phosphatide content as does a rabbit or a rat (per cc.) the plasma lecithin would have to be replenished as much as 17—22 times a day. It is significant that the high phosphatide content is maintained only during the laying period and that the red cells contain less phosphatide than the plasma, a behaviour not shown by the blood of any other animal investigated. We find furthermore that in the course of 28 hours taken by the experiment a much greater part of the phosphatides found in the plasma is labelled than of that contained in the corpuscles. This is a significant result as it demonstrates clearly that lecithin is carried to the ovary by the blood plasma and not the blood cells which obtain their lecithin in various ways. Labelled phosphatide could be taken up by the cell membrane, possibly diffuse through the cell membrane; labelled inorganic phosphorus which was found by us to diffuse at a moderate speed into the corpuscle could lead to the formation of labelled phosphatide phosphorus inside the latter, finally the lecithin could get into the corpuscles at their birth. If they are formed from labelled plasma the newly formed corpuscles should become labelled as well. As to the formation of labelled phosphatide from labelled inorganic P in blood, we found, in experiments *in vitro* that such a formation actually takes place, though only on very minute scale. As to the rate of formation of blood corpuscles, some information on this point could be obtained by injecting labelled plasma and investigating the radioactivity of the phosphorus compounds isolated from the corpuscles after the lapse of some time. If after the lapse of a day, for example, only 1% of the corpuscle phosphatides were found to be labelled we could conclude that the rate of

[1] H. E. Hamerich, *Amer. J. Physiol.* **114,** 342 (1934); S. Freeman and A. C. Joy, *loc. cit* **110,** 132 (1935).

formation of the corpuscles per day is less than 1% of the total corpuscles present.

As to the white of the egg, we find that at least a large part of its phosphorus content is drawn from organic phosphorus compounds, possibly from protein phosphorus. We arrived at this result by comparing the specific activity of the phosphorus of the white of the egg with that extracted from the shell. The latter derives its phosphate content from the inorganic P of the blood plasma and is accordingly a convenient measure of the activity of the latter. The shell is formed at about the same time as the white of the egg, the great discrepancy between the specific activity of the shell P and albumin P exclude the possibility that they are of common origin.

Summary

By administering labelled sodium phosphate to laying hens the share of the labelled phosphorus administered in the formation of the yolk, albumin and shell of the egg can be followed by aid of radioactive measurements. The comparison of the specific activity (activity per mgm P) of the phosphorus extracted from blood plasma phosphatides with that extracted from the liver, the ovary, and the yolk phosphatides leads to the result that the bulk of the phosphatides of the yolk originate in the liver. It gets from the liver into the plasma and is then taken by the latter to the ovary.

No formation of phosphatides takes place in the oviduct. After the egg leaves the ovary no more active phosphatide is formed. No formation of labelled phosphatide in the yolk can be ascertained in experiments in which an egg is placed for a day in a labelled sodium phosphate solution. In the last mentioned experiment *in vitro* slight amounts of labelled phosphorus are found in the yolk, appreciable quantities in the white, and large amounts in the shell.

The specific activity of the phosphatides extracted from the blood corpuscles was found to be only $^1/_3$ of that extracted from the plasma. Therefore, we conclude that the phosphatides formed in the liver and other organs are carried to the ovary by the plasma rather than by the corpuscles. The latter apparently play no important role in this process.

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32. THE ORIGIN OF THE PHOSPHORUS COMPOUNDS IN THE EMBRYO OF THE CHICKEN

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Several of the numerous compounds containing phosphorus present in the embryo of the chicken[1] occur in the yolk and the white of the egg. Those which do are chiefly phosphatides and nucleoproteins but, as Table 1 shows, other phosphorus compounds also occur in those parts of the egg.

Table 1. — [Plimmer ± Scott, 1909]. Percentage of the Total P (94 mgm) at the Beginning and the End of Incubation of a Hen's Egg

	Beginning	End
Inorganic P	Trace	60
Water-soluble P	6.2	8.6
Ether-soluble P	64.8	19.3
Vitellin-P	27.1	0
Nucleoprotein-P	1.9	12

Kugler [1936] has lately found that, on the twentieth day of incubation, i. e. the last day but one, only 25 mgm of the 65 mgm of lipoid P originally present in the yolk remained there; 8 mgm were found in the embryo, and the remainder had been hydrolysed yielding inorganic P. About two-thirds of the phosphatides present were found to be lecithin and one-third kephalin. In view of the large store of phosphatides present in the yolk even shortly before the egg is hatched, we should expect the embryo to avail itself of this store when it needs phosphatides to build up its nervous system and other organs containing these substances. We can test this point by introducing labelled (radioactive) sodium phosphate into the egg before incubation and investigating if and to what extent the phosphatide of the yolk and of the embryo become

[1] A detailed investigation of the soluble phosphorus compounds present in the embryo of the chicken was recently published by Needham *et al.* (1937).

labelled. If the yolk phosphatide remains unlabelled while that of embryo becomes radioactive, we can conclude that the phosphatide molecules present in the embryo have not come from the yolk but have been built up in the embryo with the participation of labelled inorganic P. Similar considerations apply to certain other compounds occurring in the embryo.

METHODS

The phosphorus content of a series of solutions is usually determined colorimetrically. For example, the inorganic P present in one sample of an acid-soluble fraction can be determined in this way, and then in another sample the phosphagen-P present can be converted into inorganic P, so that colorimetric determination now supplies the value for the inorganic P + phosphagen-P. In our experiments this was inadequate. We had to measure not only the P content but also the activity of the various fractions, so we had to obtain precipitates in each case. To obtain sufficient precipitate when dealing with eggs only incubated for a few days, it was necessary to work with several eggs simultaneously.

We precipitated the phosphorus, after bringing it into the inorganic state, as ammonium magnesium phosphate. The precipitate was then dissolved in 0.1 *N* HCl and an aliquot part was sucked into a glass cuvette. This was placed below the Geiger counter used to determine the activity of the preparations, while another aliquot part was utilized for the colorimetric determination of the phosphorus content. The glass cuvettes were covered with a thin mica window (5—6 mgm per cm^2) which only absorbed to a negligible extent the ß-rays emitted by the radioactive phosphorus; the area of the mica window was 1.1 cm^2 and the liquid content of the cuvette amounted to about 0.5 ml.

We were interested in the determination of the activity of 1 mgm P prepared from different phosphorus compounds present in the embryo or in the remains. Accordingly we were not concerned with quantitative determination of the P compounds present but concentrated our efforts on obtaining the various fractions in a pure state—to avoid, for example, traces of inorganic phosphate remaining in the phosphatides extracted from the yolk. As the phosphatides of the yolk were found to be but slightly active, while the inorganic P was strongly active, even a small contamination of the former by the latter was to be avoided. The white, the yolk, the embryo and, in some cases, the amniotic and allantoic liquids were worked up simultaneously.

As regards the white we were only interested in the total activity present after incubation. The white was ignited (reduced to ash) and its phosphorus precipitated as ammonium magnesium phosphate.

The yolk was dried with acetone and the phosphatides extracted three times from the dry product with a 3 : 1 alcohol-ether mixture. The alcohol and ether were then evaporated off at about 50° *in vacuo* and the residue was taken up with light petroleum and filtered. The filtrate was evaporated *in vacuo*, the residue ignited, and the phosphorus precipitated as ammonium magnesium phosphate.

Another part of the yolk was treated as follows. The acid-soluble compounds were extracted, then the phosphatides were removed as described above, and the residual part containing mainly vitellin-P and nucleoprotein-P was ignited; the P content of this last part was determined as ammonium magnesium phosphate.

The embryos were dropped, immediately after being removed from their eggs, into liquid air and were subsequently pulverized. The embryo powder was then extracted several times with cold trichloracetic acid—in the first two extractions a 10% solution was used, and later one of 5%. The extract was filtered into cold concentrated NaOH solution and divided into three parts, (*a*), (*b*) and (*c*). From (*a*) a sample of the average acid-soluble P of the embryo was secured, (*b*) was precipitated with 25% barium acetate solution at *p*H 6.5. The cold precipitate was washed with a dilute barium acetate solution, centrifuged and dissolved in a few drops of cold HNO_3. The inorganic P present was then precipitated by adding Fiske's reagent. The remaining filtrate was hydrolysed with *N* HCl at 100° for 7 min. to split the two labile phosphate radicals of adenosinetriphosphoric acid. The phosphorus set free was finally precipitated as ammonium magnesium phosphate. Barium hydroxide was added to the filtrate from the barium precipitation to remove any inorganic P, the precipitate was separated by centrifuging and ethyl alcohol was added to the remaining liquid until an alcohol concentration of nearly 60% was reached. The precipitate obtained after addition of alcohol [Ostern *et al.*, 1936] contained the hexosemonophosphate. Its P content was determined in the usual way. The third part, (*c*), was hydrolysed with *N* HCl in the presence of 0.1 *M* ammonium molybdate for 30 min. at 40°. In the course of 30 min. most of the phosphagen present decomposed, so that the inorganic P originally present as such, and that obtained by the decomposition of the phosphagen[1], were secured together in this fraction.

After removal of the acid-soluble P the embryo was thoroughly treated with an alcohol-ether mixture, as described above, to remove the phosphatides. The residue, containing mainly nucleoprotein-P, was ignited with concentrated sulphuric and nitric acids and the P precipitated in the usual way.

(1) On the phosphagen content of the embryo of the chicken, cf. Lehmann and Needham [1937].

RESULTS

Eggs incubated for 6—18 days. The results of the determination of the specific activities (activities per mgm P) of the different fractions extracted from seven embryos and from the remaining parts of eggs incubated for 11 days are shown in Table 2, while Tables 3—5 give the results obtained with eggs incubated for 18, 16 and 6 days. In addition to the specific activity (activity per mgm P, with that of the P extracted from the white of the egg taken as 100), we have also recorded in Tables 2 and 3 the activity (in counts per minute or in % of amount injected) and the P content of the fraction—this last quantity being determined, in all cases, by the method of FISKE and SUBBARROW.

TABLE 2. — SPECIFIC ACTIVITY OF P EXTRACTED FROM DIFFERENT FRACTIONS OF AN EGG INCUBATED FOR 11 DAYS. (SPECIFIC ACTIVITY OF P EXTRACTED FROM THE WHITE TAKEN AS 100)

	Fraction	mgm P	Counts per min	Specific activity
Embryo:	Average acid-soluble P	0.074	3.5	59
	Inorganic P	0.077	3.1	51
	Adenosine-P + inorganic P	0.121	6.0	63
	Creatine-P	0.171	8.1	60
	Phosphatide-P	0.561	29.6	67
	Residual ("nucleoprotein") P	1.49	85.6	72
Yolk:	Phosphatide-P	10.4	0.55	0.067

TABLE 3. — SPECIFIC ACTIVITY OF P EXTRACTED FROM DIFFERENT FRACTIONS OF AN EGG INCUBATED FOR 18 DAYS. (SPECIFIC ACTIVITY OF P EXTRACTED FROM THE WHITE TAKEN AS 100)

	Fraction	mgm P	% of amount injected	Specific activity
Embryo:	Average acid-soluble P	19.7	53.5	19
	Inorganic (without skeleton) P	10.91	27.2	17
	Tibia and femur-P	4.50	7.6	11
	Adenosine-P	0.048	0.14	20
	Phosphatide-P	1.08	1.7	11
	Residual ("nucleoprotein") P	0.204	0.3	10
Yolk:	Acid-soluble P	0.828	1.3	11
	Phosphatide-P	17.50	0.28	0.11
	Residual P	2.16	0.12	0.40

The figures for the specific activities (activities per mgm P) of different fractions extracted from an embryo and from the remaining parts of an egg incubated for 18 days are shown in Table 3. The P content in mgm,

the percentage of the injected activity present in the fraction and the relative specific activity are recorded; the specific activity of the P extracted from the white of the egg is taken as 100.

The specific activities obtained when the eggs were incubated for 16 and 6 days respectively are seen in Tables 4 and 5.

TABLE 4. — SPECIFIC ACTIVITY OF P EXTRACTED FRACTIONS OF AN EGG INCUBATED FOR 16 DAYS. (THE SPECIFIC ACTIVITY OF P EXTRACTED FROM THE WHITE TAKEN AS 100)

	Fraction	Specific activity
Embryo:	Average acid-soluble P	14
	Inorganic (without skeleton) P ..	14
	Tibia and femur-P	15
	Creatine-P	14
	Hexosemonophosphate-P	19
	Phosphatide-P	12
	Residual („nucleoprotein") P	16
Yolk:	Acid-soluble P	12
	Phosphatide-P	0.14
	Residual P	1.22

TABLE 5. — SPECIFIC ACTIVITY OF P EXTRACTED FROM DIFFERENT FRACTIONS OF 10 EGGS INCUBATED FOR 6 DAYS. (SPECIFIC ACTIVITY OF EMBRYO PHOSPHATIDE P TAKEN AS 100)

	Fraction	Specific activity
Embryo:	Phosphatide P	100
	Average (phosphatide) P	113
Yolk:	Inorganic P	60
	Acid-soluble minus inorganic P ..	34
	Phosphatide P	0.032
	Residual P	1.3

As the figures show, the phosphatides extracted from the yolk are only slightly active, while those extracted from the embryo show strong activity 1 mgm of embryo phosphatide-P is at least 100 times as active as 1 mgm yolk phosphatide P. Furthermore, the specific activity of the embryo phosphatide-P is about as high as that of the embryo inorganic P, showing that an inorganic P atom reaching the embryo has about the same chance of entering the skeleton as of being incorporated in a phosphatide molecule by an enzymic process—which of the two systems it enters is governed solely by probability considerations. From this it follows that the phosphatide molecules in the embryo are

not identical with those derived from the yolk, but are synthesized in the embryo.

The formation of labelled phosphatides in growing eggs was investigated by HEVESY and HAHN [1938]. It was found that the phosphatides present in the yolk are taken up from the plasma by the ovary and incorporated into the latter; as soon as the yolk leaves the ovary no more change occurs in the content or composition of its phosphatides. When labelled phosphate is administered to a hen after the yolk has left the ovary and is located in the oviduct, the egg takes up active phosphate but no active phosphatide is formed. In experiments *in vitro* as well, eggs placed in radioactive sodium phosphate solution take up active phosphate but no active phosphatides are formed. The slight activity of the phosphatides present in the yolk of incubated eggs is presumably due to the influx into the yolk of small amounts of active phosphatides synthesized in the embryo. This view is supported by the fact that the ratio of the specific activities of the embryo phosphatide P and yolk phosphatide-P was much larger (3000) in the 6 days experiment than in the 16 days experiment (100). The activity of the residual P of the yolk, which is mainly composed of vitellin and nucleoprotein, was larger than that of the phosphatides; this can be understood if we admit the possibility that the extraction of the strongly active, non-protein constituent of the yolk is not quantitative, for in this case the specific activity of the residual P would be increased.

The embryonic residue obtained after extraction of the acid-soluble and ether-soluble constituents is composed chiefly of nucleoproteins. That the specific activity of the nucleoprotein-P is the same as that of the inorganic P extracted from the embryo is not surprising, because much less nucleoprotein is present in the yolk than in the embryo (Table 1). The greater part of the nucleoproteins present in the embryo must therefore have been built up in the course of incubation; during this process labelled phosphate has an opportunity of entering the nucleoprotein molecules.

Distribution of radioactive phosphate in the egg

The greater part of the sodium phosphate injected into the white is still found at the end of the 6 days experiment in that part of the egg. The distribution of the activity between white, yolk, connecting fluids (which were not, however, free from white and yolk) and embryo is seen in Table 6.

The low activity of the yolk might possibly be due to a slow rate of penetration of the vitellin membrane by the phosphate ions; this point is under investigation. Another possible explanation is that the inorganic P content of the yolk is lower than that of the white. If a distribution

equilibrium is reached, the activity should be proportional to the amount of inorganic phosphate present in the phase in question, since the inorganic P, among all the P compounds present in the yolk and white, is practically the only source of activity; in the 6 days experiment, for

Table 6. — Distribution of Injected Active Phosphate between Different Parts of the Egg

Time of incubation	Fraction	% activity
6 days	White	61.6
	Yolk	10.3
	Liquids	26.00
	Embryo	1.7
16 days	White	14.9
	Yolk	1.7
	Liquids	19.8
	Embryo	63.0

example, 10% of the 10.3% activity found in the yolk was present as inorganic P. Finally we have to envisage the possibility that a part of the inorganic phosphate injected is not freely movable in the white—it might be precipitated as calcium phosphate or attached to proteins, its mobility being lowered thereby.

We have also carried out experiments in which 0.1 ml. physiological NaCl solution containing a negligible amount of labelled sodium phosphate was injected into eggs which were not incubated. After the lapse of 5 days the distribution of the activity in different parts of the egg was determined; 97% was found in the white and 3% in the yolk. As was of course to be expected, a still greater preference for the white was shown by the active phosphorus in this experiment; the duration of the experiment was shorter than that of those discussed above, and transport of phosphorus from the white to the embryo was absent.

To test whether the water injected encountered any hindrance in its propagation through the egg, we injected 0.2 ml. heavy water into the white of the egg; after the lapse of 5 days water was distilled separately from the white and from the yolk and the densities determined. We are much indebted to Mr O. Jacobson for carrying out the density determinations using Linderström—Lang's float method. He found that the water prepared from the white had a density exceeding that of normal water by 484 parts per million, while the corresponding figure for the water obtained from the yolk was 437. The deuterium content of the water distilled off from yolk was found to be only about 10% lower

than that of the water from the white, showing that in the course of 5 days the water injected was very nearly evenly distributed throughout the egg, in contrast to the injected active phosphate. The anomalous behaviour of the latter, while of interest in the study of the circulation of phosphate ions in white and yolk, in no way influences the investigation of the main problem discussed in this paper—namely, if and to what extent the molecules of the different phosphorus compounds present in the embryo are built up there or are drawn, from the yolk.

Introduction of labelled hexosemonophosphate into the egg to be incubated

In one set of experiments, instead of following up the fate of labelled inorganic P in incubated eggs, we introduced radioactive hexosemonophosphate. Prof. Parnas very kindly presented us with this compound (prepared by Dr Ostern) in the form of barium hexosemonophosphate, from which, by treatment with sodium sulphate in the cold, the sodium compoundof the ester was obtained. 0.2 ml., containing about 0.2 mgm P as hexosemonophosphate salt and about 3 mgm. sodium sulphate, was injected into the white of each of the eggs to be incubated; to avoid decomposition of the ester, the solution was kept ice-cooled until it was injected into the egg. Of the 10 eggs receiving this treatment, only two supplied living embryos. After a lapse of 14 days, 7.7% of the activity injected was found to have been incorporated in the embryo (5.8% in the yolk) and a large fraction was also to be found in the white and in the connecting liquids. If, of the various fractions extracted from the embryo, we had only found activity in the fraction containing hexosemonophosphate, we should have had to conclude that the hexosemonophosphate does not decompose in the egg but enters the embryo as such. In view of the results obtained in the experiments carried out with labelled inorganic phosphate, however, such behaviour was hardly to be expected. Furthermore, Kay [1926] found that in the embryo the phosphatase activity of the developing bone was extremely high, the phosphatase decomposing the hexosemonophosphate. We isolated the hexosemonophosphate from the embryo, as described on p. 295, and compared the specific activity of this fraction with that of the inorganic phosphate (+creatine-P). We also isolated the phosphatide fraction and the residual phosphorus fraction containing mainly nucleoprotein-P. As Table 7 shows, no conspicuous difference can be seen between the specific activities of the different fractions of the embryo, with the possible exception of the residual P. In these experiments small activities had to be measured and the differences found between the first three fractions lie within the errors of the experiment. The results obtained suggest the explanation that active inorganic P splits off

from the labelled hexomonophosphate injected and is incorporated in the different phosphorus compounds of the embryo, while the hexomonophosphate molecules extracted from the embryo are not those synthesized by Dr OSTERN but are molecules built up by the chicken's embryo.

TABLE 7. — SPECIFIC ACTIVITY OF P FROM DIFFERENT FRACTIONS FROM TWO EGGS INCUBATED FOR 14 DAYS AFTER THE INJECTION OF RADIOACTIVE HEXOSEMONOPHOSPHATE. (SPECIFIC ACTIVITY OF P EXTRACTED FROM THE WHITE TAKEN AS 100)

Fraction		Specific activity
Embryo:	Inorganic P	24
	Hexosemonophosphate-P	26
	Phosphatide-P	20
	Residual ("nucleoprotein") P	11
Yolk:	Inorganic P	36
	Hexosemonophosphate-P	18
	Phosphatide + residual P	0

The low value found for the residual P of the embryo may possibly be due to the building up of a part of the nucleoprotein fraction at an early date before much of the active hexosemonophosphate introduced has decomposed. The phosphatide-P and residual P extracted from the yolk were found to be inactive. These fractions were found to be only slightly active even after the injection of strongly active inorganic P, and the absence of activity after the injection into the egg of the much weaker hexosemonophosphate was only to be expected. The hexosemonophosphate fraction isolated from the yolk had a specific activity of 18; the inorganic P, 36. The larger value found for the specific activity of the inorganic P is possibly to be explained in the following way. Some active hexosemonophosphate diffuses into the yolk and partly decomposes into active inorganic P: this is the source of most of the active inorganic P which we isolated from the yolk. The hexosemonophosphate, isolated by the method outlined on p. 296, contains, besides the active hexosemonophosphate, some non-active hexosemonophosphate and possibly also some other acid-soluble P compound separated simultaneously, which diminished the specific activity of the "hexose monophosphate" fraction isolated from the yolk. In the embryo, on account of the strong enzymic action prevailing there, all phosphorus-compounds become labelled; on the other hand, in the yolk, as we have just mentioned, no such labelling takes place.

On the phosphatide synthesis in the embryo of the chicken

We saw that the phosphatide molecules present in the chicken's embryo are not identical with those formerly located in the yolk, but that they were synthesized in the embryo. The work of SCHÖNHEIMER and RITTENBERG [1936] gives us important information about the units which are utilized in the synthesis. They found, by making use of deuterium as an indicator, that the developing hen's egg forms no new fatty acids and their result excluded also the possibility that unsaturated fatty acids present in the egg had been hydrogenated during development. NEEDHAM [1931], on the other hand, found that a marked desaturation occurs in an aqueous emulsion of embryonic tissues mixed with the corresponding yolk and vigorously shaken. The embryo must thus make use of the fatty acids present in the yolk to build up its phosphatides: in doing this it possibly gives some preference to the less saturated fatty acids. The fatty acid components of the phosphatides extracted from the embryo are found to be less saturated than those extracted from the yolk residue. This, at first sight puzzling fact that the embryo, instead of using the phosphatide molecules found in great abundance in the yolk synthesizes its own phosphatide molecules, becomes less puzzling when we envisage the likely possibility that the synthesis of phosphatide molecules is a step in other chemical processes which occur simultaneously in the growing embryo.

Summary

Radioactive sodium phosphate was injected into hen's eggs which were then incubated in some experiments for 6, and in others for 11, 16 and 18 days. While the phosphatide-phosphorus extracted from the embryo always showed a high specific activity (activity per mgm P), that extracted from the yolk was hardly active at all. The phosphatide molecules present in the embryo could not therefore have been taken from the yolk only, but must have been synthesized in the embryo. The investigation of the "acid-soluble" and residual (mainly nucleoprotein) phosphorus extracted from the embryo led to a similar result—namely, that the ratio in which the labelled inorganic phosphorus atoms are incorporated into the different phosphorus compounds present in the embryo is governed solely by probability considerations. Practically all the phosphorus atoms present in the various compounds of the embryo must pass through the stage of inorganic P; only the inorganic phosphorus present in the embryo is taken as such from the yolk or the white.

In some experiments, instead of radioactive sodium phosphate, labelled hexosemonphosphate was injected into the egg before incubation. The hexosemonophosphate-phosphorus extracted from the embryo had about the same specific activity as the inorganic and the phosphatide phosporus extracted. This result suggests that inorganic phosphate radicals which were split off from the hexosemonphosphate and from other compounds present in the yolk and the white, rather than the hexosemonophosphate molecules introduced into the latter, are utilized to build up the phosphorus compounds of the chicken's embryo.

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33. FORMATION OF MILK

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We have administered labelled (radioactive) sodium phosphate to goats and investigated to what extent phosphorus present in different compounds extracted from the blood and the milk became labelled. In two cases the goat was killed after the experiment and the phosphorus compounds present in the organs investigated as well. Some of the results obtained are seen in the accompanying table.

Activity per mgm P in milk. (Activity of plasma inorg. P after 4½ hours taken as 1)			Activity per mgm phosphatide P extracted from milk and organs, after 4½ hours	
Interval after the start of the experiment	Fraction	Activity per mgm P	Fraction	Activity per mgm P
0—2 hr.	Inorg. P	0.68	Milk	0.09
	Casein P	0.54	Plasma	0.02
	Ester P	0.32	Corpuscles	0.01
			Milk gland	0.13
2—4½ hr.	Inorg. P	1.79	Liver	0.09
	Casein P	1.71	Kidney	0.11
	Ester P	1.16		
4½—6½ hr.	Inorg. P	1.71	Activity per mgm P of milk ester P accumulated in 0—3 hours	
	Casein P	1.71		
	Ester P	0.34		
23—26 hr.	Inorg. P	0.49	Hydrol. 7 min	0.76
	Casein P	0.55	Hydrol. 60 min	0.68
	Ester P	0.49	Remaining fraction	0.34
			Milk. inorg. P	1.48

Inorganic phosphorus

The inorganic phosphorus extracted from the milk produced in the first two hours after the subcutaneous injection of the labelled phosphorus, shows considerable radioactivity. Should the milk contain

only those inorganic phosphorus atoms which were located in the plasma at some time after the start of the experiment, the specific activity of the milk inorganic phosphorus should be as high as that of the plasma inorganic phosphorus. In making such a comparison, it must be borne in mind that the specific activity of the plasma inorganic phosphorus rapidly decreases with increasing time through interaction of plasma phosphate phosphorus with that of bone and other tissue. No definite conclusion can therefore be drawn from comparing a single value of the specific activity of plasma and milk phosphorus. By following up, however, the change of the specific activity of the plasma inorganic phosphorus and milk inorganic phosphorus with time, we find that it takes 3—4 hours for the milk inorganic phosphorus to be almost entirely composed of individual atoms which had been present in the plasma after the start of the experiment.

In milk produced shortly after the start of the experiment, a large part of the phosphorus atoms present were those which were located in the milk gland when the labelled phosphorus was administered. The replacement of the gland inorganic phosphorus by plasma inorganic phosphorus is thus comparatively slow because of a slow rate of penetration of the phosphate ions through the cell walls. Heavy water, on the other hand, injected simultaneously with the labelled phosphate was already, after a short time, equally distributed between plasma and milk, because of the low resistance water molecules encounter when penetrating through cell walls.

Casein phosphorus

The comparatively high specific activity of the casein phosphorus is only compatible with the assumption that the phosphorus atoms utilized in the synthesis of the casein in the milk gland are drawn from the inorganic phosphorus of the plasma. From the difference in the rates at which the active casein phosphorus and the active inorganic phosphorus present in the milk are formed, the time of formation of the casein in the gland cells can be estimated to be about 1 hour.

Ester phosphorus

The rate of formation in the milk gland of the average labelled phosphorus ester molecule is lower than that of the average casein molecule (cf. table). 1¼ hours after the administration of radioactive hexosemonophosphate (kindly presented to us by Prof. Parnas) injected into the veins of the goat, an appreciable amount of labelled ester was found in the milk, while another larger part of the activity was found in the inorganic milk phosphate. This result shows that a rapid enzymatic

breakdown of the hexosemonophosphate and rebuilding of ester molecules takes place in the gland. The milk gland contains thus enzymes having the same action on hexosemonophosphate as ROBISON and KRAY's[2] bone extracts; however, the bulk of the esters present in the milk are acted on by enzymes present in the gland at a much slower rate. Similar behaviour is shown by the mixture of phosphorus esters present in the blood[2].

Phosphatide phosphorus

The formation of active phosphatide molecules is, as seen from the table, a slow process. The individual phosphatide molecules present in the milk were mainly built up in the milk gland and not taken up as such from the plasma (as is the case with the yolk phosphatide). This follows from the fact that the specific activity of the phosphatide phosphorus extracted from the milk gland and also from the milk itself is higher than that secured from the phosphatide of the plasma. The view is often encountered that the milk fat originates from the plasma phosphatides which decompose in the milk gland, supplying fat and inorganic phosphorus. This view is entirely incompatible with the results obtained by us. To mention only one argument, we find the phosphatide phosphorus of the milk to be slightly, the inorganic phosphorus present to be strongly, active. The latter can therefore only originate from the highly active inorganic phosphorus of the plasma.

It is well known that different milk fractions, secured consecutively within a short time, have a markedly different fat content. As we find[1] that the inorganic phosphorus extracted from these fractions has a different specific activity, we have to conclude that these fractions cannot originate from an initially homogeneous liquid. So we arrive at the result that some of the milk gland cells give off milk much more readily than others, but that some even of the first-mentioned cells retain a large part of their solid milk constituents, particularly the phosphatides (and fats). Not only are phosphorus compounds present in the milk not formed during the act of milking, as often assumed, but such compounds contained in the last fraction secured during the act of milking are partly of earlier date than those present in the immediately preceding milk samples.

References

L. HAHN and G. HEVESY *Nature* **140**, 1059 (1937).
R. ROBISON *The Significance of Phosphoric Esters in Metabolism* (New York, 1932).

[1] A detailed account of the experimental results obtained will be found in the dissertation of A. W. ATEN, jun., to be presented to the University of Utrecht.

Comment on papers 27—33

In 1935, after mailing paper 16 to *Nature* we embarked on the study whether and to what extent the constituents of the brain are renewed during adult life. After the administration of ^{32}P to rats an appreciable incorporation of the tracer into brain phosphatides was observed after the lapse of 1 hr or more (paper 27). These results were published simultaneously with those of Artom *et al.* (1937) and of Chaikoff *et al.* (1937) who demonstrated the incorporation of ^{32}P into the phosphatides present in a great number of organs. We subsequently concentrated our interest on the origin of phosphatides. Among the most fascinating applications of isotopic tracers ranges the study of the origin of body constituents. With Lundsgaard we fed dogs with oil containing labelled sodium phosphate in order to find out whether an appreciable part of the increased lecithin content in the blood is built up in the intestine was labelled (paper 28). These experiments showed that intestinal mucosa is not the chief place of synthesis of plasma phosphatides. It was the results of perfusion experiments (paper 29) which first indicated that the liver releases labelled phosphatides to the circulation. In other experiments (paper 30) not the removal of the labelled phosphatides from the liver but the uptake of these from the circulation by the liver was followed. These were the first experiments in which blood containing *in vivo* synthetized labelled compounds was transfused. They led to the result that not only is the rate of turnover of phosphatides in the liver very high, but the exchange of phosphatide molecules between the liver cells and the plasma takes place at a much higher rate than the corresponding process between other organs and the circulation. The liver was found to be the main source of formation of plasma phosphatides. This was most spectacularly demonstrated by Chaikoff and his group (1946) who in the course of their very extensive and important studies on phosphatide metabolism have shown that in a hepatectomized dog, after administration of labelled phosphate, the formation of labelled plasma phosphatides practically ceases. In animals almost devoid of the higher unsaturated acids there is no diminution in the phosphatide turnover in the liver. An enhanced turnover rate is observed in the muscles of fat-starved rats (Hevesy and Smedley-Maclean, 1940).

That the chick builds up its own phosphatides and does not avail itself of the phosphatides in the yolk could be concluded from the following observation. After injecting labelled phosphate into the fertilized egg, the phosphatides extracted from various tissues of the chick were strongly radioactive while the yolk phosphatides remained inactive (paper 32).

That the phosphatide molecules of the milk in contrast to those of the yolk do not originate in the blood plasma but the former are built up in the milk gland could easily be proved (paper 33). The specific activity of the milk phosphatide phosphorus was found to amount to almost four times the specific activity of the plasma phosphatide phosphorus, but was lower than the corresponding value of the milk gland phosphatide phosphorus. The experiments were carried out at a time following administration of labelled sodium phosphate to the hen in which the activity of the plasma phosphatides was still increasing. In such experiments a milk phosphatide phosphorus specific activity which is higher than the corresponding value of the plasma phosphatides, excludes the plasma phosphatides

as a main source of milk phosphatides. A detailed description of these and a great number of additional experiments are described in the D. Sc. thesis of A. H. W. Aten Jr. (1939).

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C. Artom, C. Perrier, M. Santangello and E. Segrè (1934) *Nature* **139,** 836.
I. Perlman, S. Ruben and I. L. Chaikoff (1934) *J. Biol. Chem.* **122,** 169.
A. H. W. Aten Jr. (1939) *Isotopes and Formation of Milk and Egg.* Dissertation, Utrecht.
C. Entenman, I. L. Chaikoff and D. B. Zilversmit (1946) *J. Biol. Chem.* **160,** 5.
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34. TURNOVER OF LECITHIN, CEPHALIN, AND SPHINGOMYELIN

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Phosphatide molecules present in the body have been taken up with the food or have been built up in the organism. A spectacular proof of the synthesis of phosphatides in the body is given by the fact that ducks raised in diets containing phosphorus only in inorganic form laid 85—195 eggs during the summer[1]. These eggs contained 200—400 gm phosphatides (corresponding to 8—16 gm phosphatide P), and this very appreciable amount was synthesised by the organs of the ducks. On the other hand, phosphatides can enter the circulation from the intestine. The amount of phosphatide which is daily led by the intestinal lymph into the circulation of the rabbit[2] on normal diet was calculated to be about 50 mgm. This is only about 1/5 of the amount daily synthesised in the liver (comp. p. 323); one must further consider that an appreciable part of the above mentioned 50 mgm was synthesised in the mucosa of the small intestine. Thus, the phosphatide molecules of the organs will be only to a small extent obtained directly from the food, the overwhelming majority being built up in the body.

CONCEPT OF TURNOVER

The ultimate aim of the investigation of the origin of the phosphatide molecules present in the body is to be able to state in which form the hydrogen, carbon, nitrogen, oxygen, and phosphorus atoms present in the phosphatide molecules were taken up by the body and in what steps they were involved until ultimately incorporated into phosphatide molecules. This exacting task can hardly be solved at present, and we must content ourselves with the determination of the place and rate of formation of the phosphatide molecules in the body from glycerol,

(1) G. Fingerling, *Biochem. Z.* **38,** 448 (1911).
(2) H. Süllmann and W. Wilbrandt, *Biochem. Z.* **270,** 52 (1934).

fatty acid, choline (or another organic base), and phosphate. We will denote, in what follows, as turnover rate the rate of synthesis of phosphatide molecules from inorganic phosphate and other components independent of the actual mechanisms involved, and we shall measure this rate by determining the extent to which labelled phosphate present in the cells of an organ is incorporated into these newly formed phosphatide molecules. As the phosphatide content of an organ is usually constant, we can follow that with the formation of new phosphatide molecules the decomposition of an equal or similar number of old molecules goes hand in hand. The possibility must also be envisaged that new formation and decomposition of phosphatides do not take place in the same organ, but that the newly formed molecules are synthesised in one organ and carried into the other by the circulation.

The turnover rate can also be measured by following the rate of incorporation of fatty acids or of choline, for example, into the phosphatide molecule. The turnover rates measured by using different indicators need not necessarily be identical. It would be conceivable, for example, that the incorporation of the phosphate radical into the phosphatide molecules would be preceded by the formation of glycerophosphate and that this process would be a comparatively slow one in contrast to all other steps involved in the synthesis of the phosphatide molecule. In this case, the turnover rate measured, using labelled P as an indicator, would be slower than that found when using labelled fatty acids or labelled choline. The opposite would be the case if the reorganisation of the phosphate bond were to take place at a faster rate than the corresponding release and incorporation of fatty acids or choline into the phosphatide molecules.

The question if and to what extent the rate of phosphate incorporation into the phosphatide molecule differs, for example, from that of the fatty acid incorporation into the latter cannot be answered at the time being.

Feeding cats with mixed glyceride, the acids of which were composed to 85 per cent of elaidic acid, SINCLAIR[1] found 12 hours later the plasma phosphatide fatty acids to contain 19 per cent of elaidic acid. In our experiments we found (comp. p. 326) that, after the lapse of 16 hours, about 4 per cent of the phosphatides extracted from the plasma of rabbits contained labelled phosphate.

[1] R. G. SINCLAIR, *J. Biol. Chem.* **115**, 215 (1937).

INDICATORS APPLIED IN TURNOVER MEASUREMENTS

a) Change of the degree of unsaturation of fatty acids

Since the phosphatides contain both saturated and unsaturated fatty acids, the change of the composition of the fatty acids of the organ phosphatides after ingestion of cod liver oil, for example, can be utilised to get information on the rate of the phosphatide turnover in the organ in question. A change in the iodine number of the phospholipids extracted from the liver of dogs[1] and cats[2] after the ingestion of cod liver oil and the disappearance of the changes within 24 hours and 2 to 3 days, respectively, was observed at an early date.

b) Incorporation of iodized fatty acids into the phosphatide molecule

Iodized fatty acids, whether injected intravenously or given by mouth, enter the phosphatides of the liver, the blood[3], and the milk[4] for example,

c) Incorporation of elaidic acid into the phosphatide molecule

This method was repeatedly used in the investigation of the turnover of phosphatides. The rate of entrance of elaidic acid into and disappearance from the phosphatides was found to be rapid in the liver and the intestinal mucosa and comparatively slow in the muscle. The process was found to be essentially complete in the liver within a day, but in the muscle only after the period of many days[5].

d) Incorporation of fatty acids, labelled by introduction of heavy hydrogen, into the phosphatide molecule

Linseed oil was deuterated and the "heavy" fat obtained fed to rats. The investigation of the deuterium content of the phosphatides extracted from different organs gives information on the phosphatide turnover in the organ in question[6].

(1) G. Ioannowics and E. P. Pick, *Wien. Klin. Wochenschr.* **23,** 573 (1910).
(2) R. G. Sinclair, *J. Biol. Chem.* **82,** 117 (1929). Comp. also R. G. Sinclair, *Phys. Rev.* **14,** 351 (1934).
(3) C. A. Artom, *Arch. int. Physiol.* **36,** 191 (1933); C. A. Artom and G. Peretti, *Arch. int. Physiol.* **36,** 351 (1933).
(4) F. X. Aylward, J. H. Blackwood and J. A. B. Smith, *Biochem. J.* **31,** 130 (1937).
(5) R. Sinclair, *J. Biol. Chem.* **111,** 270 (1935); **121,** 161 (1937), M. F. Kohl, *J. Biol. Chem.* **126,** 709 (1938).
(6) B. Cavanagh and H. S. Raper, *Biochem. J.* **33,** 17 (1939).

e) Incorporation of analogues of choline, in which arsenic replaces nitrogen, into the phosphatide molecule

Arsenic can be detected in the lecithin fraction isolated from the liver and the brain of rats kept for 21 days on a diet containing arsenocholine chloride[1].

f) Incorporation of labelled phosphate into the phosphatide molecule

This method will be discussed in detail.

Most of the methods outlined above were successfully applied to show that a marked turnover takes place in some of the organs, and the application of the methods *a)*, *c)*, and *f)* lead to the result that the rate of the phosphatide turnover is much faster in the intestinal mucosa and in the liver than in the other organs. None but the "phosphate method" was applied, however, to arrive at quantitative data as to the rate of rejuvenation of the phosphatide molecules present in the different organs.

QUANTITATIVE DETERMINATION OF THE TURNOVER RATE BY USING LABELLED PHOSPHATE

The formation of phosphatide molecules containing ^{32}P inside the tissue cell can only take place when the process of phosphatide formation was preceded by a penetration of ^{32}P into the cell, and the same applies to all indicators used in turnover experiments. This point was hitherto not considered. Its great importance is best seen by the following.

To arrive at a proper figure for the turnover rate we have to compare the percentage of ^{32}P in the total inorganic P of the cells with the percentage of ^{32}P in the total phosphatide P extracted from them. If these ratios, which correspond to those of the specific activities of the inorganic P and the phosphatide P, are found to be equal, we can conclude that all phosphatide molecules were renewed during the experiment. In this case, a proportional partition of ^{32}P between the inorganic P and the phosphatide P present in the cells took place. This is only possible if the phosphate radical of all the phosphatide molecules was split off in the course of the experiment, a process which was then followed by a synthesis of phosphatide molecules with incorporation of other phosphate radicals in which $^{32}PO_4$ was represented proportionally to its total number present. If the specific activity of the phosphatide P is found,

[1] A. Welch, *Proc. Soc. Exp. Biol. Med.* **35,** 107 (1937).

to be, for example, 10 per cent of that of the inorganic P, we can conclude that 10 per cent of the phosphatides were renewed during the experiment.

Due regard must, however, be given to the change of the specific activity of the cellular inorganic P in the course of the experiment. By administering the labelled phosphate in several portions of suitably varying quantities in the course of the experiment, we can maintain a constant specific activity of plasma and interspace phosphate. As to the cellular concentration of ^{32}P, which is nought at the start of the experiment and then gradually increases, we determine the change of concentration with time experimentally and compare the specific activity of the phosphatide P extracted at the end of the experiment with the average value of the specific activity of the inorganic P which prevailed during the experiment.

When determining the specific activity of the cellular inorganic P, due regard must be taken to the fact that a part of the tissue inorganic ^{32}P is of extracellular origin. As the extracellular volume of the tissue is known and the specific activity of the extracellular P does not differ much from that of the plasma P, we can easily correct for the presence of the extracellular P in the tissue inorganic P. Since the extracellular phosphate in the case of the muscle tissue, for example, amounts to only about 1/90 of the cellular inorganic P, the correction mentioned above becomes only significant in experiments of short duration. If the rate of penetration of the inorganic phosphate differs greatly in the cells of different tissues, as it actually does, for example, in the case of the liver and the muscle, we do not get proper information on the relative rate of turnover of the phosphatides in these organs by comparing the specific activity of the liver phosphatide P with that of the muscle phosphatide P. Conclusions based on such a comparison will greatly underestimate the relative rate of phosphatide turnover going on in the muscle cells into which the inorganic P diffuses at a slow rate, in contrast to its penetration into the liver cells. We will arrive, however, at correct figures by comparing the ratio.

$$\frac{\text{specific activity muscle phosphatide P}}{\text{specific activity muscle inorganic P}}$$

with the corresponding ratio of liver products.

If we wish to draw quantitative conclusions from experiments carried out with elaidic acid as an indicator, we have to compare the elaidic acid content of the organ phosphatides with that of the elaidic acid content of the fatty acid mixture present in the corresponding cells in freely dispersed state. The latter magnitude is not known and the same consideration applies to the work with deuterated fat as an indicator.

We may get some, though very restricted, information by comparing the heavy hydrogen (D) content of the organ phosphatides with that of the organ glycerides. After the lapse of 10 hours, the ratio

$$\frac{\text{liver phosphatide D}}{\text{liver glyceride D}} : \frac{\text{kidney phosphatide D}}{\text{kidney glyceride D}},$$

where D denotes the relative heavy content of the total "non-exchangeable" hydrogen, was found to be 1 : 2.

EXPERIMENTAL PROCEDURE

The labelled phosphate of negligible weight, dissolved in physiological sodium chloride solution, was injected into the vena jugularis of the rabbit drop by drop during the experiment. Per hour 2.5 cc. were injected; the experiment took usually

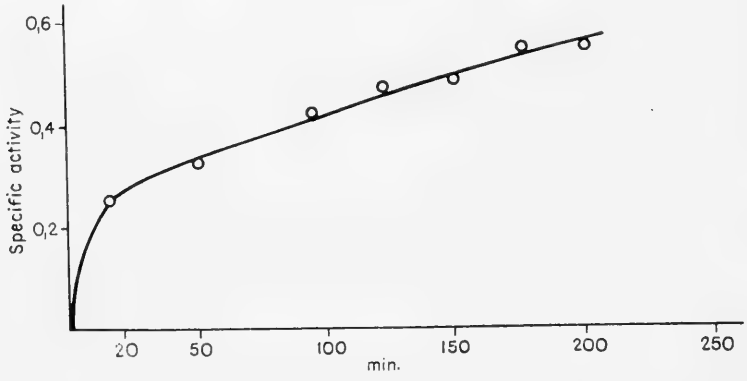

FIG. 1. Change of the specific activity of the plasma inorganic P during continuous intravenous injection of labelled phosphate to a rabbit. (Specific activity = per cent of the labelled P injected, found in 1 mgm. P).

4 hours. By taking small samples from the ear vein at different intervals, the change in the activity of the plasma was followed. In several cases, we extracted the inorganic P of the plasma and measured its specific activity (activity per mgm P), in others we contented ourselves with the measurement of the total activity of the plasma which, in experiments of short duration, is solely due to the inorganic phosphate present.

The labelled P was injected drop by drop into the vena jugularis in order to obtain a comparatively small and easily accountable change in the activity level of the plasma (see Fig. 1). If all the labelled P is injected at the start of the experiment, as in our early experiments and in all experiments carried out by other workers with labelled P, the activity level of the plasma is very high at the beginning, and it is slow at the end of the experiment (see Fig. 2). If the labelled P is given by subcutaneous injection or by mouth, the activity of the plasma first increases with time and later decreases (see Fig. 3). The sensitiveness of the radio-

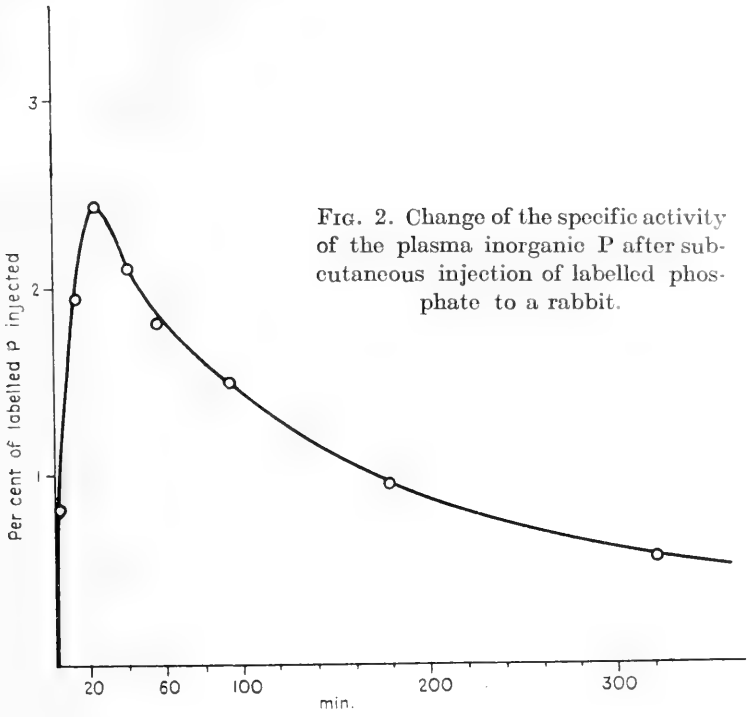

FIG. 2. Change of the specific activity of the plasma inorganic P after subcutaneous injection of labelled phosphate to a rabbit.

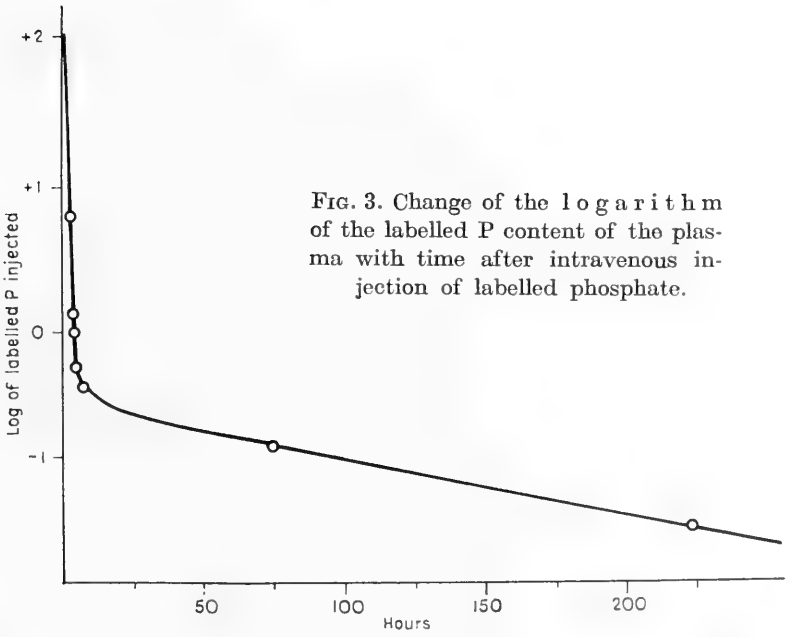

FIG. 3. Change of the logarithm of the labelled P content of the plasma with time after intravenous injection of labelled phosphate.

active indicator, thus, changes very appreciably in the course of the experiment. If we are successful in keeping the activity level of the plasma constant during the experiment, we can eliminate great difficulties otherwise encountered when calculating the turnover rate of organic phosphorus compounds.

The changes in the activity of the plasma, shown in Fig. 1, can be further reduced by injecting amounts decreasing with time. In our later experiments we have chosen this procedure and varying amounts of labelled P were adminis-

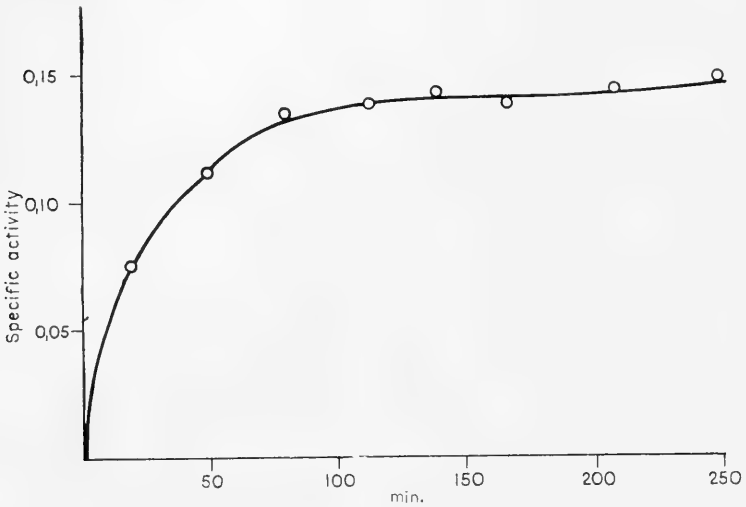

FIG. 4. Change of the specific activity of the plasma inorganic P during continuous subcutaneous injection of labelled phosphate to a rabbit. (Specific activity = per cent of the labelled P injected, found in 1 mgmP).

tered by subcutaneous injection. In an experiment taking 12 hours, for example, labelled P was injected every 20 min. In experiments taking several weeks, in the later phases of the experiment injections were made twice a day. The change in the plasma activity in such an experiment taking 4 hours is seen in Fig. 4. In experiments taking several hours or days a constant activity level could be easily obtained.

The determination of the turnover rate of the phosphatides present in the different organs necessitates the determination of the specific activity of the inorganic P and phosphatide P extracted from the organ. This determination was carried out in the following way. At the end of the experiment the animal was killed by bleeding. The organs were at once placed in liquid air, minced, and extracted with cold 10 per cent trichloracetic acid. The inorganic phosphate present was precipitated as ammonium magnesium phosphate at 0°. Muscle samples were taken before death. To secure the phosphatide present in the organs, these were first dried with cold acetone and then treated with ether, later with boiling alcohol. The ether-alcohol extracts were evaporated in vacuo and taken up several times with petrol-ether; the phosphatides were then converted into phosphate by wet ashing. The procedure applied when isolating lecithin, cephalin, and sphingomyelin will be discussed on page 330.

CALCULATION OF THE TURNOVER RATE

In most of our experiments only a minor part of the phosphatide molecules present in the organ became labelled; we can, therefore, consider the reaction leading to the formation of labelled phosphatides to be a one-sided one and disregard the decomposition of labelled phosphatides during the experiment. As already mentioned on page 312, to arrive at the value of the rate of the phosphatide turnover, we have to compare the specific activity of the phosphatide P extracted from the organ at the end of the experiment with the average specific activity of the cellular inorganic P found in the course of the experiment. The value of the activity of the cellular inorganic P is obtained from that of the tissue inorganic P after subtraction of the share due to the extracellular fluid. The correction to be applied for the presence of extracellular P in the tissue inorganic P is, in most cases, a small one. In the liver of the rabbit, for example, out of 30 mgm. inorganic P only about 0.6 mgm is located in the interspaces. We arrive at this figure by assuming that the interspaces make out[1] 22 per cent of the weight of the liver and the inorganic P content of the interspaces is 3 mgm per cent. The specific activity of the liver extracellular P is, after 4 hours, 2.5 times higher than the specific activity of the tissue inorganic P; correspondingly, 5 per cent of the total inorganic P activity of the liver is due to extracellular P.

In the case of the muscle, we arrive by an analogous consideration at the result that 25 per cent of the activity of the tissue inorganic P is of extracellular origin. The extent of the correction to be applied increases with decreasing length of the experiment, since in experiments of short duration only a small amount of labelled P penetrates into the cells.

With regard to the considerations stated above, one must recognise the possibility that some of the phosphorus which one identifies, even after the most careful experimental procedure, as inorganic P, was in fact present in the tissue in the form of very labile, not yet known, organic phosphorus compounds. Very labile P compounds of that kind, if present, would probably be in fast exchange equilibrium with the inorganic P present, and their presence would therefore not influence much the calculation given above. The labile P of adenyltriphosphoric acid comes, for example, very quickly into exchange equilibrium with the inorganic P of the tissues or the corpuscles; it is often permissible to replace the specific activity of the inorganic P by that of the above mentioned labile P. The behaviour of creatinephosphoric acid is discussed on page 325.

When calculating the turnover rate of phosphatides, we must consider the average specific activity of the cellular inorganic P prevailing during the experiment. This value is obtained by determining the specific

[1] J. F. Manery and B. Hastings, *J. Biol. Chem.* **127**, 657 (1939).

activity of the tissue inorganic P and the plasma inorganic P at different intervals. The change of the specific activity of the tissue inorganic P is seen in Table 1, that of the plasma inorganic P is discussed on page 315.

Table 1. — Specific Activity of the Organ Inorganic P as Percentage of that of the Plasma Inorganic P

Organ	100 min	240 min
Liver	12.7	42.1
Muscles	0.8(1)	4.6
Intestinal mucosa	14.7	42.8
Brain(2)	0.32	1.4
Kidneys	85	90

(1) In spite of all precautions taken, some creatine P may have been split off before the extraction of the inorganic P. The creatine P being, in experiments of short duration, less active than the inorganic P, such a decomposition may partly be responsible for the low value obtained in the experiment taking 100 min only.
(2) Comp. p. 336

It is of interest to remark that, in the case of the kidneys, after the lapse of 100 min an almost proportional partition of ^{32}P between plasma and cellular P is reached. When investigating, after 4 hours, the inorganic P of the marrow of the kidney, which makes out only a minor part of the total inorganic P of the kidney, the specific activity was found to be only 48 per cent of that of the plasma.

CELLULAR AND EXTRA-CELLULAR FORMATION OF PHOSPHATIDES

The turnover rates recorded in the fourth column of Tables 3 to 9 are calculated on the assumption that the formation of phosphatide molecules takes place inside the cells with participation of cellular inorganic P. Let us assume for a moment that the formation of phosphatide molecules takes place on the cell wall facing the interspaces. Then, not the cellular but the extracellular phosphate radicals(1) would enter the newly formed phosphatide molecules. As the specific activity of the extracellular inorganic P is often much higher than that of the cellular inorganic P, in the last mentioned case more active P atoms would take part in the synthetic process than in the first mentioned one. A high activity of the newly formed phosphatide would then not indicate such a high

(1) From this view-point, it is without any significance whether the phosphate radical is directly incorporated into the phosphatide molecule or through intermediary stages.

turnover as it would if the formation of the phosphatide molecules took place with participation of the less active cellular P. It is obvious that the sensitivity of our radioactive indicator will be very different in the two cases mentioned above. Though it is much more probable that the turnover of the phosphatide molecules takes place inside the cells we have also recorded, in the fifth column of the above mentioned tables, the turnover rates calculated on the assumption of an extracellular formation of the phosphatide molecules. The values thus obtained give the lower limit of the turnover rate, while those obtained in column 4 give

TABLE 2. — SPECIFIC ACTIVITY OF THE INORGANIC P AND PHOSPHATIDE P EXTRACTED FROM THE ORGANS

Rabbit I. — Weight: 2.4 kgm

Intravenous injection during 4 hours

Fraction	Specific activity in relative units
Plasma inorganic P	100
Liver tissue inorganic P at the end of the experiment	36.2
Liver tissue inorganic P corrected for the change in plasma activity during the experiment	44
Liver cellular inorganic P at the end of the experiment corrected as above	40.8
Liver cellular inorganic P average value during the experiment	20.4
Liver phosphatide P	3.0
Kidney tissue inorganic P at the end of the experiment	67.7
Kidney tissue inorganic P corrected for the change in plasma activity during the experiment	82.3
Kidney cellular inorganic P at the end of the experiment corrected as above	82.0
Kidney cellular inorganic P average value during the experiment	73.5
Kidney phosphatide P	5.5

the upper limit. It is conceivable that some of the phosphatide molecules are renewed inside the cell wall. In that case the inorganic P entering the newly formed phosphatide molecules will have a specific activity being the intermediary between that of the extracellular and the cellular P. A continuous drop of the specific activity of the inorganic P in the cell wall may therefore take place while the phosphate penetrates from the interspaces into the cells.

In the corpuscles the phosphatides are known to be practically concentrated in the stroma[1], and the thickness[2] of the latter to correspond

[1] B. N. ERICKSON, H. H. WILLIAMS, S. S. BERNSTEIN, J. ARVIN, R. L. JONES and J. G. MACY, *J. Biol. Chem.* **122,** 515 (1938).

[2] DANIELLI, *J. Cell. Comp. Physiol.* **7,** 393 (1936).

to that of a few molecular layers. It is, therefore, quite conceivable that in the outer layer of the stroma a slow rejuvenation of the phosphatide molecules takes place with incorporation of plasma P. In the case of finding an organic P fraction extracted from the cells or the corpuscles to show a higher specific activity than the cellular, respectively corpus cular inorganic P, we would be justified to conclude that the synthesis of the organic compound in question did not take place inside the cells, respectively the corpuscles. Investigations in the above mentioned direction may bring forward results of histochemical interest.

RESULTS OF EXPERIMENTS

Investigation of the total petrol-ether soluble phosphatide mixture

Experiments with rabbits

TABLE 3. — SPECIFIC ACTIVITY OF THE CELLULAR INORGANIC P AND PHOSPHATIDE P EXTRACTED FROM THE ORGANS

Rabbit II. — Weight: 2.6 kgm
Intravenous injection during 215 min

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	19.0	19.0	3.86
Kidney	382	18.3	4.8	3.7
Small intestine	111	7.9	7.1	1.61
Stomach	58	4.46	7.7	0.91
Heart	57.2	1.53	2.7	0.31
Lungs	66.3	4.04	6.1	0.82
Spleen	70.2	3.65	5.2	0.74
Marrow[3]	40.8	1.63	4.0	0.33
Brain	—	0.06	—	—

[1] Calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.
[3] In several experiments the specific activity of the marrow inorganic P was found to be surprisingly low, even lower than that of the ester P. These low values were presumably due to the presence of traces of only slightly active bone P in the marrow sample.

Critical remarks

In Tables 2—9, data were given for the turnover rate of phosphatides in different organs of the rabbit. When calculating those values we assumed that the labelled phosphatides present in the organs were synthesised *in situ*. In the following, we will discuss how far this assumption is justified.

TABLE 4. — SPECIFIC ACTIVITY OF THE CELLULAR INORGANIC P AND PHOSPHATIDE P EXTRACTED FROM THE ORGANS

Rabbit III. — Weight: 2.3 kgm.
Intravenous injection during 234 min

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	16.3	16.3	3.2
Muscles	7.8	0.56	7.2	0.11

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

TABLE 5. — SPECIFIC ACTIVITY OF THE CELLULAR INORGANIC P AND PHOSPHATIDE P EXTRACTED FROM THE ORGANS

Rabbit IV. — Weight: 2.5 kgm.
Intravenous injection during 215 min

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	14.8	14.8	2.9
Kidney	374	23.2	6.2	4.6
Small intestine (mucosa)	107	20.0	18.7	3.9
Heart	64.6	3.47	5.37	0.68
Lungs	76.1	7.67	10.1	1.51
Brain	—	0.175	—	—

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

Liver phosphatides

Let us first consider the liver phosphatides. Apart from the liver, an intense turnover is going on in the intestinal mucosa, and the possibility must be envisaged that the labelled phosphatides were carried into the liver from the intestine by the plasma. The plasma was found to contain only small amounts of labelled phosphatides, the specific activity of the plasma phosphatide P being, after the lapse of 4 hours, only 1/7 of that of the liver phosphatide P. This fact excludes the possibility that a substantial part of the labelled liver phosphatides was led from the intestine or any other organ into the liver. Large amounts

TABLE 6. — SPECIFIC ACTIVITY OF THE CELLULAR INORGANIC P AND PHOSPHATIDE P EXTRACTED FROM THE ORGANS

Rabbit V. — Weight: 2.1 kgm
Intravenous injection during 250 min

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	18.6	18.6	2.76
Kidney	364	22.8	6.3	3.58
Small intestine (mucosa)	115	23.6	20.5	3.54
Muscle	12.0	0.87	7.3	0.11

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

TABLE 7. — SPECIFIC ACTIVITY OF THE CELLULAR INORGANIC P AND PHOSPHATIDE P EXTRACTED FROM THE ORGANS

Rabbit VI. — Weight: 2.6 kgm
Subcutaneous injection during 255 min

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	14.8	14.8	3.2
Corpuscles	29.0	1.51	5.2	0.33

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

of water can be led from one pond into the other by a narrow channel; salt water, however, (salt corresponding to labelled phosphatides in our case) cannot pass the channel without the water of the channel becoming salt as well. The concept of "specific activity" proves, thus, to be of great use when putting forward considerations such as those discussed above.

One may say, in respect of these considerations, that, while the specific activity of the average plasma phosphatides is low, one of the phosphatide fractions (phosphatides represent a mixture of numerous compounds) might be synthesised at a very fast rate in the intestinal mucosa, and the labelled molecules formed in this process might have rushed through the plasma at a fast rate into the liver without raising much

TABLE 8. — SPECIFIC ACTIVITY OF THE CELLULAR INORGANIC P AND PHOSPHATIDE P EXTRACTED FROM THE ORGANS

Rabbit VII. — Weight: 2.4 kgm.
Subcutaneous injection during 11.5 hours

Organ	Specific activity		Percentage of phosphatides renewed during the experiment	
	Inorganic P average during the experiment	Phosphatide P at the end of the experiment	A[1]	B[2]
Liver	100	25.2	25.2	14.9
Corpuscles	25.5	4.03	15.8	2.39
Muscles	14.7	1.31	8.9	0.78
Brain	—	0.55	—	—
Marrow	36.5[3]	31.8	87.0	18.8

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.
[3] As the presence of traces only of bone P in the marrow sample investigated lowers the specific activity of the marrow inorganic P, the recorded figure for the inorganic P of the marrow may be too low and that recorded for the rate of renewal of the phosphatide P of the marrow, correspondingly, too high.

TABLE 9. — EXTENT OF RENEWAL OF PHOSPHATIDES

Rabbit IX. — Weight: 2.5 kgm.
Subcutaneous injection during 50 days

Organ	Percentage of phosphatides not renewed	
	A[1]	B[2]
Liver	0	0
Muscle	73	64
Marrow	0	0
Corpuscles	3	3

[1] Rate of renewal calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Rate of renewal calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

the specific activity of the average plasma phosphatide P. As shown on page 340, the specific activity of the phosphorus present in different phosphatide fractions can differ substantially, but, in spite of exhaustive fractionation processes no fraction of extremely high or extremely low specific activity was found. Furthermore, the total amount of labelled phosphatides formed in the intestinal mucosa in the course of 4 hours amounts to only 1/5 of that formed in the liver during the same time.

In this connection it is of interest to remark that, according to the results obtained by SÜLLMANN and WILBRANDT which are discussed on page 309, the intestinal lymph carries up to 0.1 mgm phosphatide

P[1] per hour; but, even if this amount of newly formed phosphatides is quantitatively led from the intestine into the liver, it would not suffice to account for the presence of the amount of newly formed phosphatides found in the latter which corresponds to more than 0.5 mgm phosphatide P per hour.

An entirely different argument against the intestinal origin of the labelled phosphatides found in the liver is the following. The labelled phosphatides present in the plasma were not found to leave the blood stream at a very fast rate, half of the labelled phosphatides present leaving the plasma in the course of an hour, 30 per cent of these phosphatides being found in the liver[2] thus, a rapid rush of labelled phosphatides through the plasma does not take place.

That the labelled phosphatides found in the liver are, at least to a large extent, formed *in situ*, was also shown in experiments on isolated perfused liver Such investigations were formerly[3] carried out by us on isolated cat livers in which, after the lapse of 2.5 hours, the specific activity of the liver phosphatide P was found to be about 1.5 per cent of that of the liver inorganic P. A further proof that the phosphatides present in the liver were formed there was brought about by CHAIKOFF and his colleagues[4] who found that, in experiments on rats, the removal of tissues very active in phospholipid turnover, namely the gastrointestinal tract and the kidneys, does not markedly influence the phospholipid turnover in the liver.

Muscle phosphatides

After discussing the origin of the labelled liver phosphatides we shall put forward similar arguments as to the origin of the labelled muscle phosphatides. The specific activity of the plasma phosphatides is found to be about 3 times higher after the lapse of four hours than that of the muscle phosphatides. Considerations based on the comparison of the specific activity of the plasma phosphatides and the muscle phospha-

[1] When oil is fed to the rabbit, twice that amount was found to be carried by the intestinal lymph. The feeding of oil raises the rate of turnover in the intestinal mucosa and the liver as well, as shown in experiments on rats (C. ARTOM, G. SARZANA and E. SEGRÉ, *Arch. Int. Physiol.* **47,** 245 (1938); B. A. FRIES, S. RUBEN, J. PERLMAN and J. L. CHAIKOFF, *J. Biol. Chem.* **123,** 587 (1938) and also on isolated perfused cat liver, where the turnover rate was found to be about twice as high as in experiments in which non-lipemic (normal) blood was used (L. HAHN and G. HEVESY, *Biochem. J.* **32,** 342 (1938).

[2] L. HAHN and G. HEVESY, *Nature* **164,** 72 (1939).

[3] L. HAHN and G. HEVESY, *Biochem. J.* **32,** 342 (1938).

[4] B. A. FRIES, S. RUBEN, J. PERLMAN and J. L. CHAIKOFF, *J. Biol. Chem.* **123,** 567 (1938).

tides do not, therefore, exclude the possibility that the labelled phosphatides present in the muscles were carried into them from other organs. This possibility is, however, excluded by the result of experiments based on the rate of entrance of labelled phosphatides into the muscles[1]. While, in the course of 4 hours, phosphatides showing a relative activity of 0.54 units pass from the plasma into the muscles, phosphatides having an activity of 160 units were found to be present in the muscles after the lapse of the same time.

In experiments of short duration the creatine P of the muscles gets only partly labelled and, therefore, a decomposition of creatinephosphoric acid prior to the extraction of the inorganic P will lead to a "dilution" of the activity of the inorganic P present as such in the muscle tissue. The possibility that in our experiments, taking only a few hours, too low values are obtained for the specific activity of the muscle inorganic P cannot, therefore, be entirely discarded. As the extent of the new formation of the muscle phosphatides is calculated by comparing the specific activity of the phosphatide P with that of the inorganic P, a too low value of the specific activity of the inorganic P will manifestly lead to a too high value of the rate of new formation of the phosphatides.

Kidney phosphatides

Kidney phosphatide P is found in experiments of short duration to be more active than the phosphatide P extracted from all other organs. From this fact we may, however, not follow that the kidney phosphatides are renewed at a faster rate than the phosphatides in the liver or the intestinal mucosa. The labelled inorganic P of the plasma diffuses with a remarkable speed into the kidney cells (see Table 1). This is in no way surprising in view of the role of the kidney cells as to excretion and re-absorption of phosphate. A result of this fast penetration of active phosphate into the kidney cells will be a formation of active phosphatide molecules already in the earliest stages of the experiment. This is not the case in the cells of such organs into which the labelled phosphate diffuses at a slower rate.

Labelled phosphatides of the plasma

The renewal of phosphatides in the plasma can only be determined in experiments *in vitro;* in such experiments,[2] taking 4.5 hours, the specific

[1] L. Hahn and G. Hevesy, *Nature* **144,** 204 (1939); *Kgl. Danske Vidensk. Selskab, Biol. Medd.* **15,** 6 (1940).

[2] L. Hahn and G. Hevesy, *Mem. Carlsberg* **22,** 190 (1937).

activity of the plasma phosphatide P was found to be smaller than 1/1000 of that of the inorganic P.

In experiments *in vivo*, an exchange between plasma phosphatides and organ phosphatides takes place and, as in some of the organs labelled phosphatides are formed at a fast rate, we will soon after the administration of labelled phosphate find labelled phosphatide molecules in the plasma, which were released from the organs. In fact, almost all phosphatide molecules found in the plasma were synthesized in the organs. The labelled phosphatide content of the plasma, at different times, is seen in Table 10. In this experiment, the labelled inorganic P content of the plasma was kept constant during 9 days.

Table 10. — Specific Activity of Phosphatide P and Inorganic P of the Plasma

Time	Relative specific activity	
	Inorganic P	Phosphatide P
4 hours	100	0.53
16 hours	100	3.8
25 hours	100	8.1
37 hours	100	15.0
45 hours	100	22.0
55 hours	100	27.5
9 days	100	81.6

Three consecutive processes have to precede the appearance of labelled phosphatides in the plasma. Labelled inorganic P has to diffuse into the cells of the liver and other organs in which the plasma phosphatides are formed. The building up of the labelled phosphatide molecules represents the second process, their release into the plasma the third. In view of the time taken by these processes, it is easy to understand that in the early stages of the experiment the change of the labelled phosphatide content of the plasma has a more rapid than linear dependence with time.

Since a large part of the phosphatide molecules found in the plasma originated from the liver, it is of interest to compare the amount of the active phosphatides found in the plasma with that present in the liver at the end of the experiment.

As seen in column 3 of Table 11, after the lapse of 12 hours, the activity of the plasma phosphatides reached 3/4 of that of the liver phosphatides. A large part of the liver phosphatides is, however, not yet renewed and a further substantial increase of the activity of the plasma phosphatides can only be expected by a corresponding increase in the active phosphatide content of the liver and other organs.

TABLE 11. — ACTIVE PHOSPHATIDE CONTENT OF THE LIVER AND THE PLASMA OF RABBITS

Duration of the experiment	Ratio of active phosphatide content of liver and plasma	Extent of partition of labelled phosphatides between liver phosphatides and plasma phosphatides
4 hours	94	0.16
12 hours	18	0.76
9 days	14	1.0

Phosphatide turnover in the corpuscles

Compared with the phosphatide turnover going on in the organs, the phosphatide turnover taking place in the corpuscles is but little. This is also shown by results obtained when investigating the origin

TABLE 12. — EXTENT OF PARTITION OF LABELLED PHOSPHATIDES, ORIGINALLY PRESENT IN THE PLASMA, BETWEEN THE PHOSPHATIDES OF THE CORPUSCLES AND OF THE PLASMA IN EXPERIMENTS *in vitro*

(Plasma of a rabbit containing labelled phosphatides shaken with corpuscles of another rabbit)

Animal	Time in hours	Extent of partition (Percentage)
Rabbit	0.5	1.8
	1.5	3.6
	3.0	4.0
	4.5	5.0
Hen	1.5	1.5
	2.0	2.0
	3.0	1.5

of the yolk phosphatides[1]. In these experiments, 28 hours after administration of the labelled phosphate, the specific activity of the corpuscle phosphatides was found to be only 1/3 of that of the plasma phosphatides; showing the corpuscles to be, so-to-say, a by-path of the liver and other organ phosphatides on the way through the plasma into the yolk.

The labelled phosphatide molecules of the corpuscles have various origins. Some of them were incorporated in the course of the red cell

[1] G. HEVESY and L. HAHN, *Kgl. Danske Vidensk. Selskab. Biol. Medd.* **14**, 2 (1938).

formation into a tissue containing labelled phosphatides. Some of the labelled phosphatide molecules came into the corpuscles after they reached the circulation. As seen in Table 12, in which the results of some experiments *in vitro* are recorded, a part of the phosphatide molecules of the corpuscles exchanges easily with those of the plasma. Presumably those situated in the outermost layer of the stroma take part in this exchange process. It is, however, rather difficult to interpret the comparatively high specific activity of the phosphatide P extracted from the corpuscles in experiments *in vivo* without assuming that a phosphatide turnover takes place in the corpuscles, though the rate of this turnover is small compared with that of most of the acid-soluble P compounds present in the corpuscles (see Table 14).

Table 13— Extent of Partition of Labelled Phosphatides, Originally Present in the Plasma, between the Phosphatides of the Corpuscles and of the Plasma in Experiments *in vivo*

Animal	Time in hours	Extent of partition (Percentage)
Rabbit (2—2.5 kgm)	24	16
	24	18
	24	17
	25	16
	42	34
Chicken (100—150 gm)	18	6.0
	22.5	8.1

In experiments *in vivo* with rabbits (see Table 13), in the course of a day, the activity of the corpuscle phosphatide P was found to be only about 1/6 of that of the plasma phosphatide P. A still greater difference was found when investigating chickens blood.

Using elaidic acid as an indicator, Sinclair[1] found, 8 hours after ingestion of the elaidic acid, 15 per cent of the fatty acids extracted from the plasma phosphatides to be composed of this distinctive fatty acid, while the corpuscles contained no more than traces of the indicator.

When iodised fatty acid was used as an indicator, it was found[2] not only in the phosphatides of the plasma but also in those of the corpuscles. In the latter, the concentration of iodised fat was even higher (3.3 per cent of the total fatty acids) than in the former (2.0 per cent). The application of iodised fatty acids leads, thus, to a result which is in

[1] R. G. Sinclair, *J. Biol. Chem.* **115,** 211 (1936).
[2] C. Artom, *Arch. Int. Physiol.* **36,** 101 (1933).

contradiction to that obtained by using labelled phosphate or elaidic acid as indicators. Phosphatides containing iodised fatty acids are possibly selectively taken up by the corpuscles, another explanation being that the molecules of these compounds present in the plasma were decomposed at a faster rate than those incorporated into the stroma. Phosphatides containing iodized fatty acids represent non-physiological compounds and, as shown by the above example, the results obtained by using such indicators must be interpreted very cautiously.

Table 14. — Specific Activity of Phosphatide P and Acid Soluble P of the Corpuscles

Fraction	Relative specific activity after	
	4 hours	12 hours
Phosphatide P[1]	2.6	9.6
Inorganic P	100	100
Pyrophosphate P	99.5	100
Hydrolyzed by 1 n H_2SO_4 in 7 to 100 min.	100	
Hydrolyzed in 100 min to 12 hours	100	100
Non-hydrolyzed	87	

[1] The active phosphatide molecules are partly such ones which were taken up from the plasma by a exchange process.

In this connection, the observation[1] should be also mentioned that in lactating cows during fasting a marked decrease in the concentration of plasma P lipids takes place which persists for several weeks after realimentation, but there is no significant change in the amount of red cell phosphatides. This result also shows the absence of an intense interaction between plasma phosphatides and phosphatides present in the corpuscles.

PART II

Investigation of lecithin, cephalin, and sphingomyelin

We discussed above the rate of renewal of the average petrol-ether soluble phosphatide molecules; in the following, we wish to describe some experiments in which lecithin, cephalin, and sphingomyelin were separately investigated and their turnover rate determined. Chemically, cephalin differs from lecithin by containing aminoethanol instead of choline. The biological consequence of this replacement is very significant[2]. Cephalin is highly active in accelerating blood clotting, whereas

[1] J. A. Smith, *Biochem. J.* **32,** 1856 (1938).
[2] Comp. E. Chargaff, M. Ziff and B. M. Hogg, *J. Biol. Chem.* **131,** 35 (1939).

lecithin is not. It was even reported[1] that cephalin prepared from cattle-blood or cattle-brain enhances, while lecithin inhibits the clotting of rabbits blood. The role of the phospholipids as transport agents of fats was much discussed, this role being often ascribed to lecithin alone.

In our first experiments, we determined the turnover rate of lecithin and cephalin in the organs of rabbits 4 hours after intravenous injection of labelled phosphate. We found the turnover rate of cephalin extracted from the liver, the intestinal mucosa and other organs to be pronouncedly faster than that of lecithin. Simultaneously, CHARGAFF[2] found the rate of rejuvenation of cephalin extracted from the liver and the intestinal tract of rats to be slower than that of lecithin. We were first inclined to explain this difference in the findings of CHARGAFF and ourselves by the fact that the former investigated the turnover process, in contradistinction to us, in carnivorous animals. We soon found, however, that it is the duration of the experiment which is decisive for the higher or lower rate found for the cephalin turnover. We will, in what follows, first describe the experimental procedure used.

Experimental procedure

The tissue is dried with cold acetone and extracted first with ether and then with boiling alcohol. The second process is repeated several times. The solutions obtained were evaporated to dryness and taken up by petrol-ether in the presence of pulverised dry sodium phosphate. The latter was added in order to remove traces of active phosphate possibly present. The process was then repeated in the absence of phosphate and the dry residue taken up in ether. The next step was to precipitate the cephalin from the solution by adding 96 per cent alcohol. The filtrate obtained was evaporated and the residue containing lecithin extracted with ice-cold alcohol. This procedure was repeated and the purified lecithin obtained precipitated as chloro-cadmium-lecithin. The compound obtained was thoroughly washed with ether in order to remove traces of chloro-cadmium-cephalin possibly present.

The cephalin was prepared from the alcoholic precipitate obtained in the early treatment of the phosphatide mixture. To obtain pure cephalin the precipitate was repeatedly dissolved in ether and precipitated with alcohol.

To secure sphingomyelin the fraction insoluble in petrol-ether was collected and treated alternatively with ether and ice-cold alcohol. The last residue thus obtained was dissolved in a mixture of methyl alcohol and chloroform. By adding ether to this solution purified sphingo-

[1] Y. OKARMURA, *Mitt. med. Ges. Okoyama* **48,** 1585 (1936).
[2] E. CHARGAFF, *J. Biol. Chem.* **128,** 592 (1939).

myelin was precipitated. A further purification of this product was obtained by repeating the procedure described above. When sufficient amounts were available, the sphingomyelin was recrystallised from pyridin.

Experiments with rabbits

In the experiments, the results of which are given in Tables 15 and 16, all the labelled phosphate was administered at the start of the experiment. In all later experiments, labelled phosphate was administered

TABLE 15. — SPECIFIC ACTIVITY OF INORGANIC P AND OF DIFFERENT PHOSPHATIDE FRACTIONS

Rabbit X. — Weight: 2.9 kgm

All labelled phosphate was administered at the start of the experiment by stomach tube. The animal was killed after 19 hours

Fraction	Specific activity relative to the	
	Plasma inorg. P	Inorg. P of the organ
	found at the end of the experiment	
Plasma lecithin P	39.1	—
Liver inorganic P	89.7	100
Liver lecithin P	46.3	51.6
Liver cephalin P	35.4	39.5
Liver sphingomyelin P	28.2	31.2
Brain inorganic P	4.67	100
Brain lecithin P	0.40	8.6
Brain cephalin P	1.04	22.4

TABLE 16. — SPECIFIC ACTIVITY OF INORGANIC P AND P OF DIFFERENT PHOSPHATIDE FRACTIONS

Rabbit XI. — Weight: 2.2 kgm

Labelled phosphate administered to the rabbit by subcutaneous injection at the start of the experiment. — The animal was killed after the lapse of 7 days

Fraction	Relative specific activity
Plasma lecithin P	100
Plasma cephalin P	48.1
Plasma sphingomyelin P	74.5
Corpuscles lecithin P	83.5
Corpuscles cephalin P	73.1
Brain inorganic P	26.6
Brain lecithin P	14.1
Brain cephalin P	20.9

all through the experiment to keep the specific activity of the plasma inorganic P at a constant level. In the experiments of short duration taking only 4 hours, the cephalin extracted from all the organs investigated was found to be much more active than the lecithin. While the sphingomyelin extracted from the liver did not much differ in its specific activity from that of the lecithin of this organ, in the muscle the sphingomyelin was found to be much more active than the lecithin but less active than the cephalin.

In experiments taking 12 hours, lecithin and cephalin were renewed in the liver to about the same rate while sphingomyelin was found to show a slower turnover rate. The relative activity of lecithin and cephalin was, thus, very materially different in the experiment taking 12 hours from that found in experiments of only 4 hours duration. This is not the case with the different phosphatide fractions secured from

TABLE 17. — RENEWAL OF LECITHIN AND CEPHALIN
Rabbit III. — Weight: 2.3 kgm
Intravenous injection during 234 min

Fraction	Percentage of phosphatide renewed during the experiment	
	A[1]	B[2]
Liver lecithin	10.9	2.1
Liver cephalin	27.9	5.5

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

TABLE 18. — RENEWAL OF LECITHIN, CEPHALIN AND SPHINGOMYELIN
Rabbit IV. — Weight: 2.5 kgm
Intravenous injection during 215 min

Organ	Percentage of phosphatides renewed during the experiment					
	A[1]			B[2]		
	Lecithin	Cephalin	Sphingo-myelin	Lecithin	Cephalin	Sphingo-myelin
Liver	4.38[4] / 3.67[3]	26.5	4.4	0.86[4] / 0.72[3]	5.2	0.86
Small intestine (mucosa)	17.0	40.6	—	3.55	8.47	—

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.
[3] Fraction extracted with cold ether (not protein-bound lecithin?).
[4] Fraction extracted, after removal of the ether-soluble lecithin, with hot alcohol (protein-bound?).

the muscles. In these fractions, both after 4 and after 12 hours cephalin and sphingomyelin are more active than lecithin.

When looking at the results of the experiments taking one day or more (see Tables 15, 16 and 22) we notice that the lecithin extracted from the liver is more active than the cephalin, while the opposite was found to be the case for the fractions secured from the brain.

TABLE 19. — RENEWAL OF LECITHIN, CEPHALIN AND SPHINGOMYELIN
Rabbit V. — Weight: 2.1 kgm
Intravenous injection during 250 min

Organ	Percentage of phosphatides renewed during the experiment					
	A[1]			B[2]		
	Lecithin	Cephalin	Sphyngo-myelin	Lecithin	Cephalin	Sphingo-myelin
Liver	1.4	24.6	8.95	1.86	3.68	1.34
Kidney	3.7	13.5	—	2.10	7.7	—
Muscle	2.9[4] / 1.6[3]	21.7	15.1	0.044[3] / 0.024[4]	0.33	0.23
Small intestine (mucosa)	15.6	33.4	—	2.69	5.77	—

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.
[3] Fraction extracted with cold ether.
[4] Fraction extracted, after removal of the ether-soluble lecithin, with hot alcohol.

TABLE 20. — RENEWAL OF LECITHIN AND CEPHALIN
Rabbit VI. — Weight: 2.6 kgm
Subcutaneous injection during 255 min

Fraction	Percentage of phosphatides renewed during the experiment	
	A[1]	B[2]
Liver lecithin	9.9	2.2
Liver cephalin	35.4	7.9

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

Discussion

The fact illustrated by the results described above —that in experiments taking only a few hours, the cephalin shows a higher extent of renewal than the lecithin, while, in experiments taking one day or more, the opposite is the case—suggests that not all cephalin present in the

TABLE 21. — RENEWAL OF LECITHIN, CEPHALIN AND SPHINGOMYELIN
Rabbit VII. — Weight: 2.4 kgm
Subcutaneous injection during 11.5 hours

Organ	Percentage of phosphatides renewed during the experiment					
	A[1]			B[2]		
	Lecithin	Cephalin	Sphingo-myelin	Lecithin	Cephalin	Sphingo-myelin
Liver	27.5[3] / 20.0[4]	25.9	14.8	16.3[3] / 13.4[4]	15.3	8.8
Muscle	5.6	20.6	17.2	0.49	1.81	1.51

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.
[3] Fraction extracted with cold ether.
[4] Fraction extracted, after removal of the ether-soluble lecithin, with hot alcohol.

TABLE 22. — RENEWAL OF LECITHIN, CEPHALIN AND SPHINGOMYELIN
Rabbit VIII. — Weight: 2.0 kgm
Subcutaneous injection during 9 days

Fraction	Specific activity at the end of the experiment	Lower limit of percentage renewed
Liver inorganic P	100	—
Liver lecithin P	84.0	84.0
Liver cephalin P	84.8	84.8
Muscle inorganic + creatine P	40.3	—
Muscle ester P	18.7	46.4
Muscle lecithin P	12.5	31.0
Muscle cephalin P	11	27
Muscle sphingomyelin P	16.1	40.0
Brain inorganic P	18.8	—
Brain ester P	17.3	92
Brain lecithin P	5.3	28
Brain cephalin P	5.6	30
Plasma inorganic P	100	—
Plasma phosphatide P	82	—
Corpuscle acid soluble P	94	—
Corpuscle phosphatide P	61.7	65.6

organs is renewed at the same rate, some fractions showing a much faster turnover rate than others. These fractions could differ either in their chemical composition or in their location in the cells. Numerous chemically different cephalins and lecithins exist differing, for example, in the type of fatty acids they contain. It is, however, not probable that the difference in the chemical constitution is responsible for the remark-

able difference in the turnover rate of the different cephalin fractions. The specific activity of successive fractions of cephalin crystallised repeatedly from alcoholic or other solutions does not vary appreciably (comp. p. 341). A much more probable explanation of the difference

TABLE 23. — RENEWAL OF LECITHIN AND CEPHALIN
Rabbit IX. — Weight: 2.5 kgm
Subcutaneous injection during 50 days

Organ	Percentage of phosphatides renewed during the experiment	
	Lecithin	Cephalin
Liver	100	100
Marrow	100	100
Brain	75(1) (42(2))	81(1) (46(2))
Muscle	74(1) (65(2))	71(1) (62(2))

(1) Calculated on the assumption of formation inside the cells (with incorporation of plasma inorganic P).
(2) Calculated on the assumption of formation outside the cells (with incorporation of plasma inorganic P).

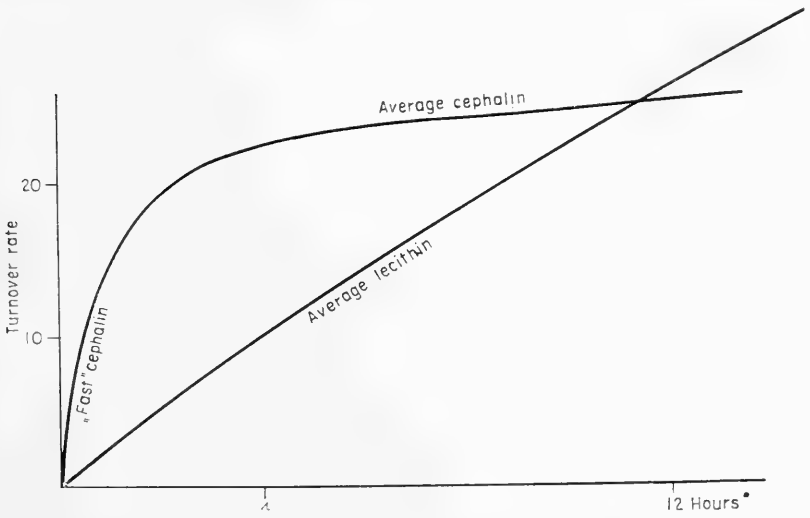

FIG. 5. Turnover of lecithin and cephalin in the liver.

mentioned above is that in some parts of the cell a decidedly more pronounced enzymatic breakdown and building up of cephalin takes place than in others. In experiments of short duration, we mainly measure the rejuvenation taking place in these favoured districts. The behaviour of lecithin is different in that we do not encounter such a pronounced variation in the rate of turnover of different fractions. The average lecithin molecule is, however, renewed at a similar rate as the average cephalin molecule. This explanation is suggested by the fact that, while

in experiments of short duration the cephalin P extracted from the liver, for example, is found to be more active than the lecithin P, in experiments of long duration both fractions are found to have about the same activity. Not only cephalin and lecithin extracted from the organs of the rabbit show this behaviour, but also the phosphatide fractions secured from the organs of rats, frogs, hens, and of isolated cat liver.

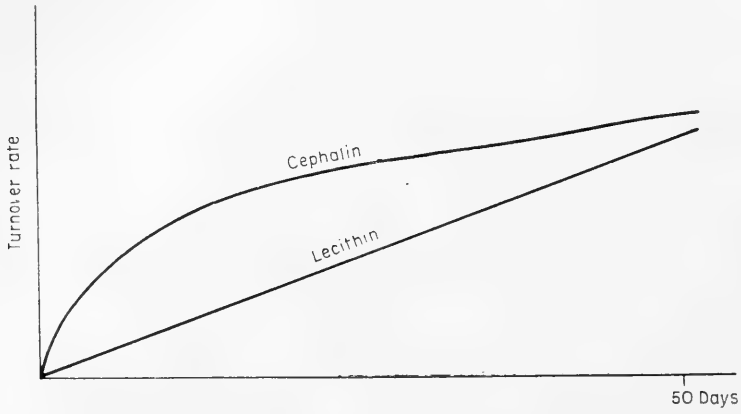

Fig. 6. Turnover of lecithin and cephalin in the brain.

That, in the case of the muscle and brain tissue, cephalin is found in experiments of long duration as well to have a faster turnover rate than lecithin is in no way in contradiction to the conclusion arrived at when investigating the liver fractions. All phosphatide fractions present in the muscle, and especially those in the brain, are renewed at a comparatively slow rate. This remark applies also to the "fast" cephalin fraction present which, though "fast" relative to the average cephalin or lecithin, is in fact "slow". This slowness has the effect that seven days do not suffice to reach the point where the amount of labelled lecithin is larger than that of the labelled cephalin. The considerations made above are illustrated by Figs. 5 and 6.

Brain phosphatide

While, in the case of other tissues, the penetration of phosphate from the plasma into the interspaces can be considered as an almost momentary process and, accordingly, the specific activity of the plasma inorganic P can be taken to be equal to that of the extracellular inorganic P, it cannot in brain tissue. We find that after 4 hours only 1/3 of the amount of labelled phosphorus (incl. organic P) is present in the brain tissue, which we should expect to be present in the extracellular space alone in case of a prompt distribution of the labelled phosphate between the

plasma and the interspaces. The extracellular space of the brain tissue was calculated from the chlorine or sodium distribution to amount to 30 per cent of the weight of the tissue[1].

TABLE 24. — FORMATION OF LABELLED PHOSPHATIDES IN THE BRAIN OF THE RABBIT

Duration of the experiment	Spec. activity of brain inorganic P / Spec. activity of plasma inorganic P	Spec. activity of brain phosphatide P / Spec. activity of plasma inorganic P	Spec. activity of brain phosphatide P / Spec. activity of brain inorganic P	Spec. activity of brain phosphatide P / Spec. activity of liver phosphatide P
215 min	0.013	0.0001	0.0093	0.0032
250 min	0.015	0.0002	0.016	0.005
11.5 hours	0.030	0.0033	0.11	0.022
9 days	0.19	0.054	0.29	0.063
50 days	0.56	0.43	0.77	0.43

In view of the foregoing statements, we cannot give exact figures for the specific activity of the extracellular and cellular inorganic P of the brain. Since these figures enter the calculation of the turnover rate of the brain phosphatides the calculation cannot be carried out. A further complication arises from the fact that the decomposition of the brain creatinephosphoric acid prior to the extraction of the inorganic P could not be avoided in our experiments. The brain creatine P may be appreciably less active than the brain inorganic P. This fact would lead to a dilution of the labelled inorganic P by non-labelled inorganic P. We record, therefore, in Table 24, *a)* the specific activity of the brain phosphatide P relative to the plasma inorganic P, *b)* relative to the brain total inorganic P, and *c)* relative to the liver phosphatide P. While the brain phosphatides are found to be much less active than the

TABLE 25. — FORMATION OF LABELLED LECITHIN AND CEPHALIN IN THE BRAIN OF THE RABBIT

Duration of the experiment	Relative specific activity		
	Plasma inorganic P	Brain lecithin P	Brain cephalin P
250 min	100	0.0092	—
11.5 hours[1]	100	0.25	1.08
19 hours[1]	100	0.40	1.04
50 days	100	42	46

[1] In this case, the total activity was injected at the start of the experiment.

[1] J. F. MANERY and B. HASTINGS, *J. Biol. Chem.* **127**, 657 (1939).

liver phosphatides, high values are obtained for the ratio of the specific activity of the brain phosphatide P and the brain inorganic P. Even if we divide these values by 2, to account for the diluting effect of the creatine[1] P, the resulting figures will still be high.

CHANGUS, CHAIKOFF and RUBEN[2] observed a progressive increase in the content of radioactive phosphatides in the brain on rats for about 200 hours after the administration of labelled phosphorus and it is of interest to note that, in a recent investigation, CHAIKOFF and his colleagues[3] found that the specific activity of the phosphatide P is not uniform throughout the central nervous system.

Experiments with rat

The specific activity of lecithin P and cephalin P expected from the rat's liver is given in Tables 26 and 27.

While the ratio of the specific activity of cephalin and lecithin P was found, after 3 hours, to be 1.33, after 24 hours we find the value 0.7. Similarly CHARGAFF[4] found, in experiments taking 24 hours, greater

TABLE 26. — SPECIFIC ACTIVITY OF LECITHIN AND CEPHALIN IN THE RAT'S LIVER
Weight of the rat: 200 gm
All labelled phosphate was injected subcutaneously at the start of the experiment; 190 min later, the rat was killed

Fraction	Percent of activity injected, found in 1 mgm phosphatide P
Liver lecithin	0.21
Liver cephalin	0.28

turnover figures for lecithin than for cephalin. He found the above ratio to be 0.8. It is also interesting to note that an early paper of ARTOM and his colleagues contains data on the relative activity of lecithin and cephalin extracted from the liver of rats to which olive oil and labelled sodium phosphate was administered 9 hours previously. They state the above ratio to be about 0.6.

[1] C. ARTOM, C. PERRIER, M. SANTANGELLO, G. SARZANA and E. SEGRÉ, *Arch. Int. Physiol.* **45,** 35 (1937).

[2] G. W. CHANGUS, J. L. CHAIKOFF and S. RUBEN, *J. Biol. Chem.* **126,** 493 (1938).

[3] B. A. FRIES, G. W. CHANGUS and J. L. CHAIKOFF, *J. Biol. Chem.* **132,** 24 (1940).

[4] E. CHARGAFF, *J. Biol. Chem.* **128,** 592 (1939).

TABLE 27. — SPECIFIC ACTIVITY OF DIFFERENT P FRACTIONS IN THE RAT'S LIVER
Weight of the rat: 222 gm
All labelled phosphate was injected subcutaneously at the start of the experiment; 24 hours later, the rat was killed

Liver fraction	Relative specific activity[1]
Labile P	100
Lecithin P	117
Cephalin P	82
Non-labile acid-soluble P	103
Protein P	31

[1] The figures are given relative to the labile acid-soluble P, the value of which, after 24 hours, closely corresponds to that of the inorganic P.

Experiments with frogs

The turnover figures of cephalin and lecithin extracted from the frog's liver were found, as would be expected, to be lower than the corresponding figures found in experiments with mammalia. The specific activity of the cephalin P was found to be much higher than that of the lecithin P.

TABLE 28. — SPECIFIC ACTIVITY OF DIFFERENT P FRACTIONS IN THE LIVER OF A FROG
Labelled phosphate was injected into the lymph-sack of a frog kept at 20° all through the experiment (4 hours)

Liver fraction	Relative specific activity
Inorganic P	100
Cephalin P	7.8
Lecithin P	1.3

Ratio of specific activity of cephalin and lecithin = 6.

Experiments with laying hens

That, in a laying hen, the specific activity of cephalin P of the liver is found, after the lapse of 5 hours, to be slightly higher than that of lecithin P, while in experiments with rabbits a very great difference was found, is just the result which we have to expect in view of the arguments discussed on p. 335. In the course of 5 hours, the phosphatide molecules present in the liver of the hen are renewed to an extent which in the case of the rabbit is first reached after the lapse of many hours.

TABLE 29. — SPECIFIC ACTIVITY OF P FRACTIONS IN THE ORGANS OF A HEN WEIGHING 900 GM
Labelled phosphate was administered to a laying hen by subcutaneous injection at the start of the experiment; the hen was killed 5 hours later

Fraction	Relative specific activity
Plasma lecithin P	1.00
Plasma cephalin P	0.98
Liver lecithin P	2.76
Liver cephalin P	2.93
Liver sphingomyelin P	1.38
Liver protein P	0.15
Kidney lecithin P	1.15
Kidney cephalin P	1.69
Intestinal mucosa lecithin P	0.90
Intestinal mucosa cephalin P	1.05
Intestinal mucosa sphingomyelin P	1.10

It is, therefore, not surprising that the fractions obtained from the hen's liver are similar to those secured from the rabbit's liver in experiments of much longer duration. In the kidneys of the laying hen the phosphatide molecules are renewed at a slower rate than in the liver and, in this organ, as was to be expected, cephalin is found to be markedly more active than lecithin.

The liver sphingomyelin of the laying hen which does not enter the yolk to any appreciable extent is renewed at a decidedly lower rate than the petrol-ether soluble phosphatides. It is also interesting to note that the rate of rejuvenation of the protein P in the liver of the laying hen is about 20 times slower than that of the phosphatide P.

In the intestinal mucosa, cephalin and sphingomyelin are formed at a somewhat higher rate than lecithin. In the kidneys cephalin was found more active than lecithin. That the rate of renewal of phosphatides in the liver of laying hens is decidedly higher than in the intestinal mucosa or other organs was also found in our earlier researches.[1]

Experiment with perfused cat liver

The experiment on cat liver which was carried out with the kind help of Professor LUNDSGAARD also indicates the faster cephalin turnover in experiments of short duration. The fasting cat used in this experiment weighed 3.3 kgm. Blood circulated for 70 min through the isolated liver.

[1] G. HEVESY and L. HAHN, *Kgl. Danske Vidensk. Selskab, Biol. Medd.* **14**, 2 (1938).

Besides labelled phosphate of negligible weight, 500 mgm alcohol was added to the blood at the start of the experiment and 500 mgm glycine after 30 min.

When fractionating the alcoholic solution of the liver cephalin, the less soluble fraction was found to show the higher specific activity amounting

Table 30. — Specific Activity of P Fractions in the Liver of a Cat
Duration of experiment: 70 min.

Fraction	Relative specific activity
Plasma inorganic P	100
Plasma lecithin P	0.18
Liver lecithin P	2.43
Liver cephalin P	4.05

Ratio of the activity of cephalin P and lecithin P = 1.67.

to 4.47. The low lecithin activity of the plasma is, in view of the short duration of the experiment, not surprising. The labelled phosphate requires some time to penetrate into the liver cells, the formation of labelled lecithin takes some time as well and, finally, the release of the phosphatides into the plasma is far from being a momentary process.

Survey of the results

In the course of 4 hours, an appreciable part of the petrol-ether soluble phosphatides present in the intestinal mucosa and the liver were found to be renewed. This result is in conformity with that found by Artom and his colleagues[1], by Chaikoff and his collaborators[2], and in this laboratory[3]. In Tables 31 and 32, a summary of the data obtained on the renewal rate of lecithin, cephalin and sphingomyelin fractions is given. In Table 32, the very different behaviour of lecithin from cephalin is clearly seen. While, in the case of lecithin, the labelled percentage increases more or less linearly with time, this is far from being the case with cephalin. We find an almost linear increase with time in the amount of labelled lecithin formed in the liver and the muscles, assuming that the formation of this compound takes place inside the cells. This linearity does not hold if we assume the formation of phosphatides to take place with incorporation of extracellular P. The bulk of the labelled liver

[1] C. Artom, C. A. Perrier, M. Santangello, G. Sarzana and E. Segrè, *Arch. Int. Physiol.* **45**, 32 (1937).

[2] B. A. Fries, S. Ruben, J. Perlman and J. L. Chaikoff, *J. Biol. Chem.* **123**, 587 (1938).

[3] L. Hahn and G. Hevesy, *Nature* **144**, 204 (1939).

TABLE 31. — EXTENT OF RENEWAL OF THE PETROL-ETHER SOLUBLE PHOSPHATIDE MIXTURE EXTRACTED FROM THE ORGANS OF THE RABBIT IN THE COURSE OF 4 HOURS
The results are computed from the figures of Tables 2—7.

Organ	Percentage of phosphatides renewed during the experiment	
	A[1]	B[2]
Small intestine (mucosa)	19.6	3.7
Liver	16.7	3.1
Lungs	8.1	1.2
Stomach	7.7	0.9
Muscle	7.3	0.11
Kidney	6.2	4.3
Spleen	5.2	0.74
Corpuscles	5.2	0.33
Heart	4.0	0.50

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

lecithin could not be formed in the last mentioned way, since in that case (see column 5 of Table 32) nine times as much labelled lecithin should have been formed in the course of 12 hours than was found after 4 hours. Similar considerations apply to the muscle lecithin where in the course of 12 hours seventeen times as much labelled lecithin should have been formed as after 4 hours. Such an increase with time is highly improbable.

If we consider the two possibilities of the formation of cephalin, i. e. incorporation either of cellular or of extracellular labelled inorganic P, we arrive at the following result. If the labelled cephalin is formed inside the liver cells, as much as 1/4 became labelled within 4 hours; thus, 1/4 of the total cephalin present undergoes a rapid renewal, the remaining 3/4 being comparatively inert. In the course of the following 8 hours, hardly any further increase of the amount of newly formed cephalin can be noticed. That the remaining part of the cephalin is also renewed, though at a very slow rate, is, however, shown by the fact that, after 9 days, most of the cephalin present at the start of the experiment was found to be labelled. Muscle cephalin behaves in an analogous way.

If we now consider the possibility that the labelled cephalin is formed with incorporation of extracellular P, we arrive at an entirely different interpretation of the results. The amount of labelled liver cephalin formed in the course of 12 hours then works out to be about three times that formed during 4 hours. This result is quite plausible. The result obtained in the case of the muscle cephalin, where as much as five times more labelled cephalin should have been formed in the course of 12

TABLE 32. — EXTENT OF RENEWAL OF LECITHIN, CEPHALIN AND SPHINGOMYELIN IN THE ORGANS OF THE RABBIT IN THE COURSE OF EXPERIMENTS LASTING 4 HOURS AND 12 HOURS, RESPECTIVELY

The results are computed from the figures in Tables 17—21.

Organ	Percentage of phosphatides renewed					
	A[1]			B[2]		
	Lecithin	Cephalin	Sphingo-myelin	Lecithin	Cephalin	Sphingo-myelin
	after 4 hours					
Small intestine (mucosa)	16.3	37	—	3.1	7.1	—
Liver	9.3	28	6.7	1.7	5.6	1.1
Muscle	2.3	21	15.1	0.03	0.33	0.23
Kidney	3.7	13	—	2.1	7.7	—
	after 12 hours					
Liver	25	26	15	14.6	15.3	8.8
Muscle	5.6	21	17	0.5	1.8	1.5

[1] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of cellular inorganic P.
[2] Turnover rate calculated on the assumption that the formation of phosphatides took place with incorporation of extracellular inorganic P.

than in 4 hours, seems less plausible. While it is not probable that the cephalin present in the liver should have been formed with incorporation of extracellular inorganic P, we must envisage the possibility that a part of the cephalin located in the cell membranes is renewed with incorporation of inorganic P located inside the membrane. We discussed above the two extreme cases, formation of phosphatides with incorporation of cellular and of extracellular P. While penetrating through the cell wall, the inorganic P may experience a more or less continuous drop in its activity and the renewal of phosphatide molecules located in the cell membranes could take place by incorporation of "intermediary" labelled phosphate radicals.

It is of interest to remark that in a laying hen, where the liver has to supply large amounts daily of both lecithin and cephalin, the "slow" cephalin fraction is also renewed at a remarkable rate and the rejuvenation of the average lecithin and cephalin in the course of 5 hours hardly differs.

Difference between "fast" and "slow" cephalin

That the organs contain a small cephalin fraction which is renewed at a fast rate and a larger one which is slowly renewed may possibly be due to a difference in the chemical composition of these fractions. Since

different cephalin fractions obtained by fractional crystallisation of the total cephalin extracted from the organ in question did not show large variations, it is not probable that the above result can be explained as due to different rates of new formation of cephalins of different chemical composition.

In fractional crystallisation of alcoholoc cephalin solutions, only minor differences in the specific activity of the fractions were noticed. The least soluble fraction extracted from the liver showed, for example, a turnover rate of 4.47, while the value found for the average fraction was 4.05. When organs were extracted first with ether and then with hot alcohol, the lecithin prepared from the first extract was found to be somewhat more active than was the lecithin prepared from the alcohol extract (see Table 19).

Since the renewal of cephalin is an enzymatic process, its velocity should be determined by the effectivity of the enzymes present. It is probable that that part of the cephalin which is located in such a region of the cells, where the enzymatic action is very pronounced, is renewed at a very fast rate. It is also probable that this "fast" fraction has a different biological significance from the "slow" fraction. The fact that the phosphatides have a much larger turnover in some organs than in others induced SINCLAIR[1] to distinguish between metabolic and non-metabolic phosphatides. The former ones found in the liver, for example, should be involved in fat metabolism; the latter ones, found for example, in the muscle, should play an important role in building up cell membranes. Our results suggest the interpretation that we have in all the organs nvestigated a "fast" and a "slow" cephalin fraction as well. The "fast" fraction is the smaller one. To what extent the "fast" cephalin and other phosphatide fractions are involved in fat metabolism is under investigation.

Summary

Labelled sodium phosphate was administered to rabbits, rats, frogs and laying hens. In order to keep the concentration of the labelled phosphate in the plasma constant, labelled phosphate was injected from time to time throughout the experiments.

The specific activity of the inorganic P extracted from the plasma and the organs was measured at intervals. From these data the average specific activity of the cellular inorganic P prevailing during the experiment was calculated.

The phosphatides present in various organs were extracted as well, and the specific activity of the phosphatide P and also of the lecithin, cephalin and sphingomyelin P determined.

The knowledge of the average specific activity of the cellular inorganic P during the experiment and that of the phosphatide P at the end of the experiment

[1] R. G. SINCLAIR, *Physiol. Rev.* **14**, 357 (1934).

permits us to calculate the extent of new formation (turnover) of the phosphatides on the assumption that this process takes place inside the cells. In case the phosphatide molecules are renewed with incorporation of extracellular inorganic phosphate, the specific activity of the latter enters the calculation.

The specific activity of cephalin P extracted from different organs was found in experiments of short duration (4 hours) to be much higher (up to 10 times) than that of lecithin P. With increasing time of experiment this difference was found to diminish. In the fractions obtained from the rabbits liver, after the lapse of 12 hours, both fractions showed the same activity. In organs like muscle and brain, in which a slow phosphatide turnover takes place, an equal activity of lecithin and cephalin is only reached after the lapse of several days.

Sphingomyelin is renewed in the liver at a slower rate than the ether-soluble phosphatides. In the muscles, in experiments taking not longer than 12 hours, sphingomyelin was found to be appreciably more active than lecithin, but less active than cephalin; after the lapse of 9 days, sphingomyelin was found to be the most active fraction.

Two alternative explanations are put forward to explain the difference in the behaviour of cephalin and lecithin : (a) a part (about 1/4) of the cephalin present inside the cells is renewed at an appreciably higher rate than the average cephalin present, while the bulk of the cephalin showed a similar turnover rate as the average lecithin; or (b) a part of the cephalin located in the cell walls is renewed *in situ* with incorporation of inorganic phosphate which has a higher specific activity than the inorganic P located inside the cells.

In the course of 50 days, all phosphatide molecules present in the liver and the skeleton were found to be renewed. However, only 74 per cent of the lecithin and 71 per cent of the cephalin extracted from the muscles were newly formed in the course of the experiment. In the brain tissue, 1/4 or more of the lecithin and 1/5 or more of the cephalin molecules remained unchanged.

The amount of active lecithin and cephalin present in the plasma and corpuscles was determined. The active plasma phosphatide molecules are not formed in the circulation but in the organs and are led into the circulation. Most of the phosphatide molecules present in the corpuscles were incorporated during the formation of the erythrocytes, but some turnover takes place inside the corpuscles.

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35. TURNOVER OF PHOSPHATIDES

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SINCE the time when it was first shown that ^{32}P administered to animals is easily incorporated into liver phosphatides (ARTOM *et al.*, 1937; HAHN and HEVESY, 1937; PERLMAN *et al.*, 1937) and the first attempt to arrive at a quantitative figure for the rate of renewal (turnover) of liver phosphatides (ARTOM *et al.*, 1938; HEVESY and HAHN, 1940 a), the problem of phosphatide turnover in the animal organism has found an ever-increasing interest. The above-mentioned observation was not surprising in view of the early observation by ARTOM (1933) that iodinated fatty acids, when administered, promptly enter the phosphatides of the liver, of SINCLAIR's (1936) similar observation on feeding elaidic acid, and the still earlier work by IVÁNOWICS and PICK (1910) on the change in iodine number of phosphatides extracted from the livers of dogs after ingestion of cod liver oil.

In later studies with ^{32}P, liver phosphatides were found to be the almost sole source of plasma phosphatides[1], to interchange readily between liver and plasma, and also to be metabolized to a large extent in the liver. Furthermore, numerous investigations were carried out with the aim to elucidate the effect on phosphatide formation by feeding choline, cholesterol, amino acid, etc. Surveys of these investigations were recently given by CHAIKOFF and ZILVERSMIT (1948) and by HEVESY (1948).

PRECURSOR OF PHOSPHATIDE PHOSPHORUS

The application of isotopic indicators in turnover studies often aims at the determination of the average life-time of a type of molecules in an organ. We wish, for example, to know the time during which the

[1] ARTOM *et al.* (1948) demonstrated recently that fed intact labelled phosphatide molecules can be absorbed into the circulation. These absorbed phosphatide molecules were found, however, to account for a minor part only of the total amount of ^{32}P fed as phosphatide P.

average phosphatide (or lecithine and so on) molecule in the liver remains unchanged. The occurrence of a change may involve certain moieties of the molecule only or a reassembly of all the moieties or, finally, the molecule may leave the organ unchanged, as do some of the phosphatide molecules of the liver, which interchange with phosphatide molecules of the plasma. In the last mentioned case, the determination of the rate of renewal (replacement) of the phosphatides does not encounter difficulties. When replacing part of the plasma of one animal by plasma of another animal, containing ^{32}P labelled phosphatides, we can follow the rate of disappearance, thus the rate of replacement of plasma phosphatides. In the first mentioned cases we may, however, encounter great difficulties due to our ignorance of the steps involved in the biosynthesis of most types of molecules. We can, however, determine the upper limit of the time during which the average phosphatide molecules of the liver, for example, remain unchanged by administering labelled phosphate and comparing the specific activity of the phosphatide P at the end of the experiment with the specific activity of the intracellular inorganic P which prevailed in the average during the experiment. In experiments of short duration, the repeated renewal of a phosphatide molecule may usually be neglected. Is that not the case, a more complicated calculation has to be used, as will be shown below (cf. p. 360).

The rate of incorporation of labelled intracellular orthophosphate may indicate the rate of renewal of the phosphate group of the phosphatide molecule, it will, however, not necessarily do so. If first with the participation of orthophosphate, or with a phosphorus compound which comes into rapid exchange equilibrium with orthophosphate at a comparatively slow rate, a formation of phosphorus containing precursor of the phosphatide molecule takes place, and this formation is followed by a relatively rapid incorporation of the precursor into the phosphatide molecule, the following up of the rate of incorporation of the intracellular orthophosphate into the phosphatide molecules fails to reveal the rapid interchange taking place between the ultimate precursor and the phosphatide molecule.

It is quite possible that the labelled orthophosphate is incorporated into the phosphatide molecule during the assembly of the molecule and that thus orthophosphate or a phosphorus compound which comes into rapid exchange equilibrium with orthophosphate as ATP is not only an early, but also the last precursor of phosphatide P. It is, however, equally possible that glycerophosphate or another organic P compound is the last precursor of phosphatide phosphorus.

Following a single administration of labelled phosphate the specific activity of the liver intracellular orthophosphate first increases; in the later phase of the experiment, however, when the decrease in specific activity of the plasma inorganic P results in a partial exodus of ^{32}P

from the liver cells into the plasma, the specific activity of the liver orthophosphate P decreases. While, in the first phase of the experiment, with increasing time an increasing incorporation of ^{32}P takes place into liver phosphatides, in the second phase of the experiment, the already labelled phosphatide molecules are continually renewed and, as this renewal takes place in a medium of decreasing activity, the specific activity of the phosphatide P also decreases. The slower the organic compound is labelled in the first phase of the experiment, the slower it will lose its activity in the second phase, as was already found in early experiments with phosphatides and will be discussed below. A precursor will thus have a higher specific activity than the product formed in the early phase of the experiment and a lower one in the second phase. To these two criteria an important third was added by ZILVERSMIT and assoc. (1943), viz. that the maximum specific activity of the precursor P must coincide with the beginning decrease in specific activity of the phosphatide P. Often the practical application of this "Zilversmit criterium" encounters difficulties. Based on cumulative evidence including specific activity-time relations, ZILVERSMIT and assoc. (1948) arrive, however, to the result that it is likely that glycerophosphate is the pertinent precursor of liver lecithin.

We shall in the following mainly consider the rate of incorporation of orthophosphate, which has the same specific activity as the labile phosphate of ATP, into phosphatides. Even though the consideration that orthophosphate P is the precursor of phosphatide P, leads to a lower limit of the turnover rate only, it involves many advantages which one appreciates when faced with the task to calculate the turnover rates in investigations in which labelled carbon, nitrogen, sulphur, etc. are applied as indicators.

EXTRACELLULAR AND INTRACELLULAR ORTHOPHOSPHATE

It is probable (SACKS, 1949) that orthophosphate participates in phosphorylation processes during its passage through the cell boundary. It is likely that ^{32}P reaches the cells as ATP P_3 or ATP $P_{2,3}$ and then gets into rapid exchange equilibrium with the cellular orthophosphate.

The importance of the intracellular orthophosphate P as a precursor —even if it were not the ultimate one—is enhanced by the fact that within a very short time intracellular orthophosphate P and ATP P_3 (and to a large extent even ATP P_2) show the same activity level. This is of interest both in view of the fact that ATP is an important phosphate donor and also because the specific activity of ATP P_3 is a more reliable measure of the specific activity of the genuine intra-

cellular orthophosphate than the value obtained in the direct determination of tissue orthophosphate, even if the extraction was preceded by viviperfusion.

In the case of the rat liver, which is showing both a high capillary wall and a high cell membrane permeability, we found in all our experiments lasting 2 hours to 84 days within ± 10 per cent identical values between the specific activities of tissue orthophosphate P and ATP P_2, P_3, indicating that in these cases it is not advantageous to consider the specific activity of ATP P_2, P_3 instead of the corresponding value of the tissue orthophosphate P. In experiments, however, in which the formation of labelled phosphatides in the mouse liver was investigated, only 10 min following intravenous injection of labelled phosphate, a marked difference between the specific activity values of orthophosphate P and ATP $P_{2,3}$ was found. Taking the specific activity of the plasma orthophosphate P to be 100, the corresponding values of liver orthophosphate P and ATP $P_{2,3}$ were found to be 31.9 and 21.5, respectively. Assuming the extracellular volume (25 %) of the liver to have the same concentration (5 mgm%) and specific activity as the plasma orthophosphate P, we find in 100 gm liver 1.3 mgm extracellular orthophosphate P having an activity of $1.3 \times 100 = 130$, while the total tissue orthophosphate P, the concentration of which is 32.3 mgm%, has an activity of 1030; thus, in this case, the activity of the extracellular orthophosphate P makes out 13 % of the activity of the total liver orthophosphate. In experiments of such short duration it is advisable to consider the specific activity of ATP $P_{2,3}$, or preferably that of ATP P_3, as a measure of the specific activity of the intracellular orthophosphate P.

As another case in which the specific activity of ATP $P_{2,3}$ has to be regarded as a measure of the specific activity of the intracellular orthophosphate P, that of the lung may be mentioned. One hour after administration of labelled phosphate by subcutaneous injection to 170 gm rats, the relative specific activity values (the plasma orthophosphate value being taken = 100) for the tissue inorganic P and ATP $P_{2,3}$ were found to be 52.7 and 35.6, respectively, the higher value of the specific activity of the tissue inorganic P being possibly due to the presence of highly active extracellular orthophosphate P in the tissue orthophosphate investigated.

CALCULATION OF THE RATE OF RENEWAL

In all previous calculations, except a quite recent one to be discussed on p. 358, carried out hitherto it was assumed that labelled phosphate, or another precursor which comes into rapid exchange equilibrium (compared with the rate of formation of the labelled phosphatides)

with the labelled phosphate, is incorporated into the labelled phosphatides. If this assumption, holds and 1 mgm inorganic intracellular liver phosphate contains throughout the experiment 1000 ^{32}P atoms, the presence of 10 ^{32}P atoms in 1 mgm of phosphatide P at the end of the experiment, lasting for example 1 hour, indicates that 1 per cent of the phosphatide molecules present in the liver is formed during the experiment. This figure indicates the percentage labelled molecules. As some of the molecules will be turned over twice or even several times during the experiments, and the formation of one labelled phosphatide molecule from another labelled phosphatide molecule is not registered by this method, the number of phosphatide molecules turned over in 1 hour will be greater than 1 per cent. If the average concentration of the labelled molecules was 0.5 per cent during the experiment, about 1 per cent of this 0.5 per cent would be "invisibly" renewed a second time.

The total percentage of phosphatide molecules turned over will thus be 1.005 per cent. The effect of more than 2 renewals of the same molecule in experiments of restricted duration can be disregarded and the same applies, in experiments of short duration, even for the second renewal of a molecule, as the percentage error of this type of experiments is quite appreciable. In the recent work by Bollman and Flock (1948), for example, in which remarkably uniform results were obtained, the deviations of the specific activity values obtained for both the inorganic P and the phosphatide P are still of the order of $\pm$ 10 per cent.

In view of this fact an exact evaluation of turnover rates is often without interest. However, if such an evaluation is required, one is tempted to take into account, beside the repeated renewal of the phosphatide molecules in the course of the experiment, the constant dilution of the labelled molecules by non- (or slightly) labelled ones which penetrate from the plasma into the liver. In the dog, 2 per cent of the liver phosphatides were found by Zilversmit *et al.* (1943 b) to be replaced by plasma phosphatides in the course of 1 hour, a somewhat greater replacement being found by Hevesy and Hahn (1940 b) in the liver of the rabbit. Assuming the liver of the rat to show a similar behaviour to that of the dog, about 0.1 per cent of the phosphatide content of the liver will be replaced by plasma phosphatide molecules in the 1 hour-experiment.

In our considerations we assumed the specific activity (activity per mgm P) of the orthophosphate P of the liver to remain constant during the experiment. This is not strictly correct. In former experiments (Ahlström and assoc., 1944) the mean value of the specific activity of the orthophosphate P of the rat liver during a 2 hour-experiment was found to be about 5 per cent larger than the end value. In

recent experiments we found this figure to be about 10 per cent. In these experiments the labelled sodium phosphate was administered by subcutaneous injection. If, however, the labelled phosphate is given by intravenous injection, as in some of the experiments of BOLLMAN *et al.* (1948), a greater difference, viz. 24 per cent, is found between the mean and the end value of the specific activity of the orthophosphate P. While subcutaneous injection is followed by an increase in the specific activity values of the plasma inorganic P during the first phase of the experiment followed by a comparatively slow decrease, intravenous injection is followed by a rapid decrease in the specific activity of the plasma inorganic P which is the precursor of the liver inorganic P. Intravenous injection is thus a less favourable procedure when we aim at a constant activity level of the orthophosphate P of the liver in 2 hour-experiments. In experiments like the last mentioned one, and also when maximum precision is wanted, even in the first mentioned type of experiments we have to take into account the change of the specific activity of the orthophosphate P during the experiment.

The percentage ratio of the specific activities of the end value of the phosphatide P and of the mean value of the orthophosphate P during the experiment indicates the lower limit of the percentage of new phosphatide molecules (new as to their P content) formed during the experiment. As already mentioned above, if we want to know the percentage of phosphatide molecules turned over during the experiment, we must consider among others a repeated renewal of the same molecule as well and the percentage ratio of the mean specific activities of the phosphatide P and the inorganic P during the experiment. In most experiments of restricted duration, for example in our experiments taking 2 hours, the correction due to the repeated renewal lies well within the errors of the experiment.

In experiments of long duration in which a large percentage of the phosphatides is renewed, the calculation carried out by ZILVERSMIT *et al.* (1943 a) can be used with advantage. This calculation is based on the change of the specific activity of the phosphatide P with time; specific activity values at different times have thus to be known as well as the mean value of the specific activity of the inorganic P during the experiment. While the last mentioned method is generally applicable, the first mentioned method can only be applied advantageously when the percentage turned over is not over 20—30 per cent. Its advantage is that the knowledge of one phosphatide activity value, that determined at the end of the experiment, suffices to calculate the turnover rate. If a correction of the repeated renewal of the same molecule is wanted, it suffices to assume that the mean value of the specific activity of the phosphatide P during the experiment is half of its end value.

FORMER INVESTIGATIONS

In the first investigations (HEVESY and HAHN, 1940) in which the specific activity of the plasma inorganic P of the rabbit was kept constant during an experiment lasting 4 hours, the percentage renewal of the phosphatides with incorporation of intracellular orthophosphate or an organic phosphate which comes into rapid exchange-equilibrium with the intracellular orthophosphate, was found to be 16. In the experiments by HAHN and TYRÉN (1946) the mean value of the specific activity of the intracellular orthophosphate P of the liver was not determined. A comparison of the values obtained for rabbit and rat livers shows, however, that phosphatides are turned over at an appreciably more rapid rate in the liver of the rat. In experiments of 2 hours' duration, HEVESY (1947) found a percentage renewal with incorporation of intracellular orthophosphate of 7.6 per hour for phosphatides of the whole liver tissue of the rat and a value of 4.5 only for the percentage renewal

TABLE 1. — CALCULATION OF BOLLMAN ET AL.'S EXPERIMENTAL DATA OF THE TURNOVER OF LIVER PHOSPHATIDES

Duration of experiment in hours	Percentage renewal per hr		
	Calculated according to ZILVERSMIT *et al.*	Calculated according to HEVESY and HAHN	
		Without considering repeated renewal	Considering repeated renewal
0.5	4.95	4.89	4.95
1	5.17	4.04	5.16
2	5.34	6.08	5.34
4	4.93	4.39	4.93[1]

(1) If, instead of considering the known mean phosphatide specific activity during the experiment, we consider $^1/_2$ of the end value, 4.83 is obtained.

of the phosphatides present in the cell nuclei. BOLLMAN *et al.* (1948) arrived at a figure of 5.3 for the percentage renewal of the liver phosphatides in the course of 2 hours. These authors raise doubts as to the applicability to their case of the method of calculating the turnover rate as outlined above (HEVESY and HAHN, 1940 a) and perform their calculations by applying the method worked out by ZILVERSMIT *et al.* (1943). That the discrepancy between the percentage turnover of the liver phosphatides of the rat as found by BOLLMAN *et al.* and by the present authors is not due to a difference in the method of calculating the experimental figures obtained, is demonstrated in Table 1, in which the results of the evaluation of the experimental data

of BOLLMAN *et al.* are calculated both according to their and our method, almost identical figures being obtained in both cases.

The method of calculating the experimental results can thus not be responsible for the difference in the renewal percentage obtained by different workers and we have thus to consider other explanations.

As shown in the present communication, the ratio of the specific activities of the orthophosphate P and phosphatide P of the liver varies with the age of the rat, and these variations can be assumed to be at least partly responsible for the discrepancies mentioned above[1].

EXPERIMENTAL

To each of more than 100 rats of known age, kept on normal diet, 0.1 ml physiol. NaCl solution containing ^{32}P of 2 μcurie activity and a negligible ^{31}P content was administered by subcutaneous injection. Two hours later, batches of 4 or more rats were pooled, the animals were killed by decapitation, bled, and the isolated organs frozen with solid CO_2. An aliquot of the liver, spleen and kidney samples was used to determine the specific activity of the inorganic P, another for the total P, while a third was used for the determination of the specific activity of phosphatide P. In the case of the liver, the labile P of ATP was investigated as well. All organs were cut into small pieces and average fractions were obtained. To measure the inorganic P values, fractions (0.2—0.3 gm) were extracted with cold CCl_3COOH. The total P was obtained by wet ashing of about 0.2 gm fresh tissue, while a few gm were used for the extraction of phosphatides.

Before extracting phosphatides the tissue was treated with 200 ml acetone for 15 minutes. The filtrate was dried in a CO_2 atmosphere and the residue extracted with ether. The acetone treated tissue was extracted by grinding it in a mortar twice with 150 ml ether and once with 1 : 3 ether-alcohol mixture for 15 minutes. The residue was then extracted for 8 hours in a Soxhlet flask with 150 ml boiling ether-alcohol mixture (1 : 3). All ether and alcohol fractions were united and dried in a CO_2 atmosphere. To free the phosphatides from traces of inorganic P and other P compounds the residue was dissolved in 300 ml ether and shaken with 450 ml 0.1 n HCl + 0.01 n NaCl solution in a separating funnel. This procedure was repeated four times, as suggested by HAHN and TYRÉN (1945).

The ethereal solution was evaporated in a Kjeldahl flask and then ashed by a mixture of H_2SO_4 and HNO_3. An aliquot of this solution was used for colorimetric determination and another precipitated as magnesium ammonium phosphate and its radioactivity determined.

To determine the specific activity of the labile P of ATP about 8 gm of liver tissue were extracted with 3 volumes cold CCl_3COOH solution. The cooled filtrate was neutralized to phenolphtalein with cooling by adding solid $Ba(OH)_2$.

The precipitate containing adenosine triphosphate, adenosine diphosphate, orthophosphate, and some other minor fractions of organic P compounds was washed with a little $Ba(OH)_2$ and neutralized with CCl_3COOH. Subsequently it was dissolved in 15 ml n HNO_3. To the solution, as suggested by SACKS and ALTSCHULER (1942), NH_4NO_3 was added until a concentration of 5 per cent was

[1] That the spec. activity of the liver P of mice declines from 3.7 to 1.6 when the age increases from 6 to 24 weeks was observed by FALKENHEIM (1943).

obtained, followed by 2 ml 10 per cent ammonium molybdate solution. The inorganic P was precipitated overnight; the filtrate was then hydrolyzed for 20 minutes at 100°C and cooled. The precipitate contained the labile P of ATP. This was dissolved in 15 ml 5 per cent NH_3 and its P precipitated as magnesium ammonium salt. The precipitate was dissolved in 0.1 n HCl, an aliquot being used in the colorimetric essay, while another was precipitated as magnesium ammonium salt and reserved for the radioactive measurements.

Percentage Turnover of Liver Phosphatides

The lower limit of the percentage turnover of liver phosphatides per hour, which is calculated from the percentage ratio of the specific activities of the liver phosphatide P at the end of the 2 hour-experiment and the mean value of the orthophosphate P during the experiment (which was by 10 per cent less than the end value) and divided by 2, is given in column 2 of Table 2, while column 3 contains values

Table 2. — Lower Limit of the Percentage Turnover per Hour of the Phosphatides of the Rat Liver. ATP $P_{2,3}$ Scale

Age of rats	Percentage turnover per hour	
	Without considering repeated renewal	Considering repeated renewal
4 d	12.1 ± 1.2	12.7 ± 1.3
10 d	9.9 ± 1.0	10.4 ± 1.1
14 d	10.0 ± 1.0	10.5 ± 1.1
30 d	9.6 ± 1.0	10.1 ± 1.0
10 d	7.7 ± 0.8	8.1 ± 0.8
1.5 year[1]	5.7 ± 0.6	6.0 ± 0.6

[1] Calculated from inorganic P value.

corrected for the repeated renewal of phosphatide molecules during the experiment. The correction is obtained by calculating the percentage ratio of the average specific activities of the phosphatide P and the orthophosphate P during the experiment. The average ratio of the specific activity of the phosphatide P was taken to be half of the end value (cf. footnote, p. 353). The turnover rate is seen to decrease with the age of the rats, the percentage turnover rate of the phosphatides of 1.5 year-old rats being only about half of that obeserved in 4 day-old rats.

In Table 3 the percentage turnover calculated by comparing the specific activity of the phosphatide P with that of the inorganic P is shown.

Bollman *et al.* (1948) found the percentage turnover per hour to be about 5. From the fact that their rats weighed 200 gm we have to conclude that the animals investigated were fully grown rats for which we arrive at a corresponding figure of 8 to 6. Furthermore, we have to

consider that in contrast to the present authors, they investigated fasting rats. PLATT and PORTER (1947), when comparing the turnover of phosphatides in the liver of fed and fasting rats, found the former value to be about $^1/_3$ larger than the latter. That the diet influences the percentage renewal of liver phosphatides of the rat was also shown recently by CAMPBELL and KOSTERLITZ (1948).

While we cannot calculate turnover rates from the ratio of the specific activities of the phosphatide P and total liver P, this ratio indicates to what extent phosphatide P atoms are renewed compared with the average total P atoms. We listed these ratios in Table 4 along with the total P content of the livers investigated. We have not listed the inorganic P nor the phosphatide P contents as we were interested primarily in phosphatide P and inorganic P fractions of high purity and the extended purification processes entailed an appreciable loss.

The absolute amount of phosphatides renewed during a given span of time increases, in contrast to the percentage renewal, with the age of the rat, as both the weight of the liver and its phosphatide content are increasing with age. The phosphatide content of the liver of the

TABLE 3. — LOWER LIMIT OF THE PERCENTAGE TURNOVER PER HOUR OF THE PHOSPHATIDES OF THE RAT LIVER. INORGANIC P SCALE

Age of rats	Percentage turnover per hour	
	Without considering repeated renewal	Considering repeated renewal
4 d	10.2 ± 1.0	10.7 ± 1.1
10 d	9.8 ± 1.0	10.3 ± 1.0
14 d	10.4 ± 1.0	10.9 ± 1.1
90 d	7.9 ± 0.8	8.3 ± 0.8
1.5 y	5.7 ± 0.6	6.0 ± 0.6

TABLE 4. — SPECIFIC ACTIVITY OF LIVER PHOSPHATIDES AS PERCENTAGE OF THE SPECIFIC ACTIVITY OF THE TOTAL P OF THE LIVER

Age of rats	Percentage specific activity	Total P of liver in mgm p.c.
4 d	67	324
10 d	64	318
14 d	74	363
30 d	63	348
90 d	43	374
1.5 y	58	370

90-day-old rat (3200 mgm%) is $^4/_3$ times that of the 4-day-old rat (2800 mgm%) and the weight of the liver increases simultaneously from 0.24 gm to 4.0 gm (LANG 1937). The phosphatide content of the 90-day-old rat is thus 20 times that of the 4-day-old rat. The percentage turnover of the 4-day-old rats being 1.5 times that of the 90-day-old animals, the amount turned over in the course of 1 hour in the 90-day-old animals is

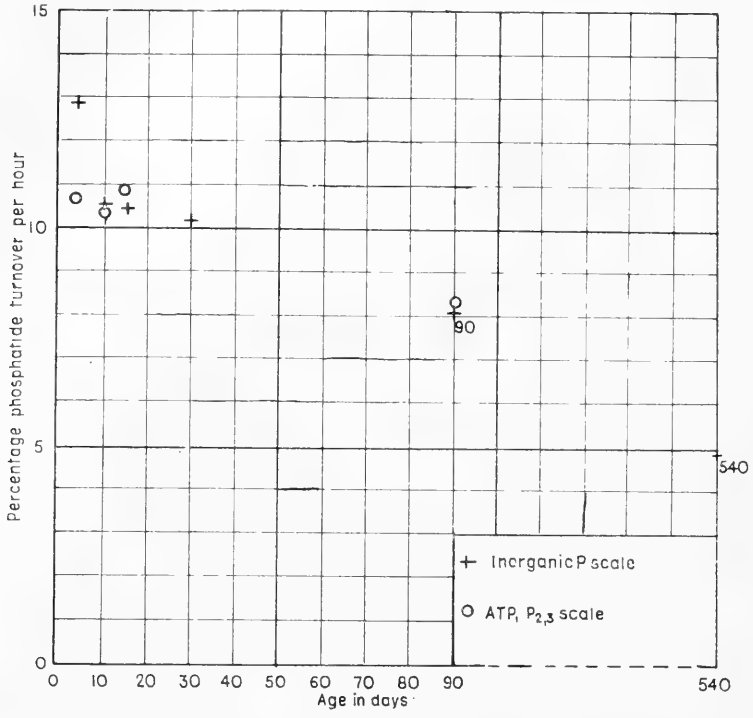

FIG. 1. Percentage incorporation of orthophosphate P resp. ATP $P_{2,3}$ per hour into liver phosphatides of rats of different age

15 times that renewed in the 4-day-old rats, the figures being 10.25 mgm. and 0.69 mgm, respectively. If between the phosphatide and the orthophosphate (or ATP) molecule a phosphorus compound were interposed which was formed at a comparatively slow rate, it would be the ultimate precursor of the phosphatide molecule; then the above mentioned figure would represent the lower limit of the amount of phosphatides turned over only.

Spleen Phosphatides

Since we do not know the mean value of the orthophosphate P of the spleen during the experiment we cannot calculate the lower limit of turnover rate. Assuming the mean value to be $^2/_3$ of the end value

for the 90-day-old rat, we arrive at a lower limit of percentage turnover of 3 per hour by making use of the data given in Table 5. This is a rough estimate which indicates that the turnover rate is about $^1/_3$ of that found for the liver phosphatides.

Table 5 contains data of the relative specific activities of the spleen total P and phosphatide P and the plasma inorganic P, the spleen inorganic P value being taken to be = 100.

TABLE 5. — SPECIFIC ACTIVITY OF SPLEEN PHOSPHATIDES
EXPERIMENT TAKING 2 HOURS

Age of rats in days	Percentage ratio of the specific activity of phosphatide P to that of		
	Spleen inorganic P	Spleen total P	Plasma inorganic P
4	4.8	13.7	4.9
10	5.2	17.0	4.8
14	5.0	18.3	—
30	3.9	18.5	3.4
90	3.8	13.8	2.3
540	3.4	16.0	1.6

TABLE 6. — SPECIFIC ACTIVITY OF KIDNEY PHOSPHATIDES
EXPERIMENT LASTING 2 HOURS

Age of rats in days	Percentage ratio of the spec. activity of phosphatide P to that of		
	Kidney inorganic P	Kidney total P	Plasma inorganic P
10	14.0	44.0	10.3
14	14.4	37.2	10.6
30	15.2	41.3	13.5
90	12.1	27.9	11.2
540	12.1	37.8	11.6

The percentage ratio of the specific activities of the phosphatide P and spleen inorganic P measured at the end of the experiment decreases with the age of the rat, the decrease being more pronounced if we consider the ratio of the percentage activities of the phosphatide P and plasma inorganic P, as seen in Table 5. This decrease indicated a decreasing permeability with age of the spleen cells to inorganic P, a fact previously observed by AHLSTRÖM *et al.* (1944) and by ANDREASEN and OTTESEN (1945).

Kidney Phosphatides

The ratio of the specific activities of kidney phosphatide P and inorganic P is appreciably higher than the corresponding spleen values, though it falls below the liver values. We found the mean value of the specific activity of the kidney inorganic P to be 1.35 times the end value and, consequently, we have to divide the figures of column 3 of Table 6, which denote data of experiments, taking 2 hours, by 2×1.35 in order to arrive at an estimate of the lower limit of the percentage turnover per hour.

The pronounced decrease in the phosphatide turnover with age shown by the liver phosphatides is not exhibited by the kidney phosphatides.

Turnover of Lecithin and Cephalin

In the investigations described above the rate of incorporation of ^{32}P into the total liver phosphatides was determined. Lecithin and cephalin are renewed at a not very different rate. Platt and Porter (1947) found, for example, 6 hours after administration of labelled phosphate to full-grown rats kept in usual diet the ratio of specific activities of lecithin P and cephalin P to be 1.5, while in the fasting rat 1.3 was found. These authors state, furthermore, that administration of choline increases the turnover rate of lecithin while ethanolamine promotes the turnover of cephalin formation. In the first mentioned case a maximum increase of 33 per cent, in the latter case a maximum increase of 87 per cent of the turnover rate was observed. They interpret the increased formation of labelled phosphatides following administration of choline and ethanolamine, respectively, as a mass action, the assembly of the phosphatide molecule being promoted by an increase in the choline and ethanolamine concentration, respectively.

In experiments on dogs, Zilversmit and assoc. (1948) recently found the mean specific activity ratio of lecithin P and cephalin P to be 1.2.

Table 7. — Specific Activities

Fraction	Specific activity[1]
Plasma orthophosphate P	0.546
Liver orthophosphate P	0.550
Glycerophosphate P	0.376
Total P	0.180
Total tissue phosphatide P	0.0705
Mitochondria phosphatide P	0.0473
Cell nuclei phosphatide P	0.0296

[1] Activity of 1 mgm P in percentage of the activity administered to the rat.

Phosphatide Turnover in Cell Nuclei and Mitochondria

The figures obtained by different investigators for the turnover rate of phosphatides (or for the turnover rate of lecithin, cephalin) are average figures, as the turnover rate in different types of cells and even in different parts of the same cell (cf. HEVESY, 1947) differ. In Tables 7 and 8 the specific activities of the various isolated fractions resp. the percentage replacement of phosphatide P of the total liver tissue of the cell nuclei and of the mitochondria of the liver by tissues inorganic P, glycerophosphate P and total liver, resp., P is stated. Isolating glycerophosphate we made use of the method applied by ENTENMAN *et al.* (1948). 6 μcurie of ^{32}P were administered by subcutaneous injection to each of 6 rats weighing about 150 gm. The animals were killed after the lapse of 2 hours.

The phosphatide P turnover of mitochondria makes out 67% only of the corresponding figure of the total phosphatide P while the corresponding figure for the cell nuclei is 42 only. The last mentioned figure was in a former investigation found to be 65. The discrepancy is due to the fact that in the method used formerly of separation of cell nuclei (DOUNCE, 1945) the nuclei containing fraction contained also mitochondria, in which as seen above, phosphatides are turned over at a more rapid rate than in the cell nuclei.

The activity of 1 mgm phosphatide P in percentage of the activity of 1 mgm liver orthophosphate P listed in Table 8 indicates the lower limit of the percentage replacement of phosphatide P in the course of the experiment taking 2 hours. By a fortuitous coincidence, the end value and the average value, of the specific activity of liver orthophos-

TABLE 8. — PERCENTAGE REPLACEMENT OF THE PHOSPHATIDE P OF THE LIVER

Phosphatide fraction	Activity of 1 mgm phosphatide P in percentage of the activity of 1 mgm P of			
	Plasma orthophosphate	liver ortophosphate	liver glycero-phosphate	liver total P
Total tissue	13.4	12.8	18.75	39.2
Mitochondria	9.37	8.95	13.1	27.5
Cell nuclei	5.64	5.38	7.89	16.5

phate are almost identical in an experiment taking 2 hours. This is not the case for the specific activity of glycerophosphate P, as the labelled glycerophosphate accumulates gradually in the course of the experiment by incorporation of labelled orthophosphate P. We can assume the average value of the specific activity of glycerophosphate P during the

experiment to be about half its end value. Correspondingly, we have to multiply the figure 18.75 listed in Table 8 by 2 to arrive at the percentage renewal of the liver phosphatides, assuming glycerophosphate to be the last phosphatide precursor; when, on the other hand, assuming orthophosphate P to be a relevant precursor, a value of 12.8% is obtained. The turnover rate calculated on glycerophosphate "basis" is thus about 3 times the value obtained, supposing that orthophosphate P is the relevant precursor.

In experiments on dogs, ZILVERSMIT *et al.* (1948) found a similar ratio (3.5) for the turnover rate of liver lecithin calculated on the assumption that glycerophosphate P resp. orthophosphate P is the ultimate phosphatide P precursor. Assuming glycerophosphate to be the precursor of phosphatides, the turnover time of the liver phosphatides of the 150 gm rat works out to be 5 hours.

As stated on p. 359, several arguments were put forward in support of the view that glycerophosphate is the ultimate precursor of phosphatides and that the comparison of the specific activity of the liver phosphatide P with the corresponding value of the liver glycerophosphate P supplies a 3 times as high, and correct, turnover rate, as does comparison with the liver orthophosphate P. This would indicate that it is the formation of labelled glycerophosphate which takes comparatively long time, while the incorporation of labelled glycerophosphate into

TABLE 9. — EFFECT OF FEEDING OF LABELLED GLYCEROPHOSPHATE RESP. LABELLED ORTHOPHOSPHATE ON THE FORMATION OF LABELLED PHOSPHATIDES IN THE LIVER OF THE RAT

(ARTOM and SWANSON 1948)

Specific activity (arbitrary units)	Labelled glycerophosphate fed	Labelled orthophosphate fed
Liver glycerophosphate P	45.3	18.0
Liver orthophosphate P	21.6	23.4
Liver phosphatide P	8.1	6.9
Plasma phosphatide P	0.5	6.8

the phosphatide molecules is performed at a comparatively rapid rate. This conclusion is not easy to reconcile with a recent finding by ARTOM and SWANSON (1948). These authors state that 6 hours following feeding of labelled glycerophosphate, the specific activity of liver glycerophosphate P was much greater than the corresponding value of orthophosphate P of the liver and also much greater than the orthophosphate P value of the liver following feeding of labelled sodium phosphate. The specific activity of phosphatide P of the liver was, however, not significantly greater in these experiments in which a high liver glycero-

phosphate specific activity was observed. The presence of a highly active glycerophosphate in the liver did thus not markedly accelerate the formation of labelled phosphatides. The above stated specific activity figures were found by ARTOM and SWANSON 6 hours after feeding of the labelled compounds.

In view of the fact that the results of only one experiment is stated, the results of this investigation, which aimed at the elucidation of a very different problem, can however not be considered to invalidate ZILVERSMIT *et al.* conclusions. A closer investigation of the phosphatide formation in the liver following feeding of labelled glycerophosphate would not be without interest.

Search for the Existence of a Small Phosphatide Fraction of Rapid Turnover Rate

It is conceivable that a small fraction of phosphatides is present in the liver, which is renewed at a more rapid rate than the average phosphatide molecule present. The existence of such a fraction could influence much the conclusions drawn in the last paragraph which are based on the number of phosphatide molecules turned over during a time unit.

TABLE 10. — SPECIFIC ACTIVITIES OF P FRACTIONS OF MICE 10 MINUTES FOLLOWING INTRAVENOUS INJECTION OF ^{32}P

Fraction	Specific activity[1]		
	1	2	3
Plasma orthophosphate P	20.4	32.6	31.8
Liver orthophosphate P	6.53	—	6.56
Liver ATP $P_{2,3}$	4.30	4.26	—
Liver phosphatide P	0.0983	0.0898	0.0813

[1] Activity of 1 mgm P in percentage of the activity administered.

Let us assume that of 100 phosphatide molecules 99 are renewed to an extent of 18% in 1 hour, while 1 phosphatide molecule is renewed 100 times during the experiment. Our experiments would hardly reveal the presence of such a fraction responsible for 118 renewal processes, while our turnover experiments would reveal a 19% renewal of the average phosphatide molecules, only. One hundred renewals per hour is a large figure in view of the fact that the very rapidly rejuvenated ATP $P_{2,3}$ in experiments *in vitro* is found to be renewed 72 times per hour only (MEYERHOF *et al.*, 1938). But even a 1% phosphatide fraction renewed 10 times in the course of 1 hour would be responsible for 10 renewal processes beside the 18 performed by the rest of 99% phosphatide molecules, increasing the number of molecules turned over to 28.

The presence of a small rapidly renewed fraction should become noticeable, in experiments of very short duration.

We carried out experiments in which about ½ microcurie ^{32}P was injected into the tail vein of each group of 10 mice; the (22—26 gm) animals were killed by decapitation after the lapse of 10 minutes, the organs pooled, and the specific

activity of the plasma orthophosphate, liver inorganic P, liver ATP $P_{2,3}$ and phosphatide P determined. Some of the results obtained are listed in Table 10. The phosphatides were purified according to Levin's method as modified by Hahn and Tyrén (1946). The above results do not indicate the presence of a rapidly renewed phosphatide fraction.

Experiments of Long Duration

As observed at an early date (Hevesy and Aten, 1938; Zilversmit *et al.*, 1943, 1948) in experiments of long duration the specific activity of phosphatide P exceeds the corresponding value of orthophosphate P, the last mentioned magnitude decreasing at a slower rate than the orthophosphate P. As seen in Table 11 the specific activity of orthophosphate P resp. labile ATP phosphorus declines between $^1/_{12}$ day and 84 days (the maximum value is observed about $^1/_{12}$ day after subcutaneous injection) from 0.546 to 0.000603. Thus, out of 900 ^{32}P atoms in the

Table 11. — Specific Activity of Liver Fractions at Different Dates Following Administration of ^{32}P

Fraction	Specific activity[1] after					
	1/12 day	1 day	12 days	21 days	44 days	84 days
Orthophosphate P ...	0.546	0.108	0.0153	0.00849	0.00134	—
ATP $P_{2,3}$	—	0.105	0.0185	0.00481	—	0.000603
Phosphatide P	0.00703	0.121	0.0252	0.00518	0.00169	0.00066

[1] Activity of 1 mg P in percentage of the dose administered, obtained by comparing the radioactivity of a known aliquot of the solution injected with that of a known amount of phosphorus of the fraction investigated.

nitial maximum state, only 1 is present after the lapse of 84 days (beside the loss due to radioactive decay). During the same interval, the beginning of which does not correspond, however, to the maximum value of ^{32}P content of phosphatides, the ^{32}P content of phosphatides decreases to $^1/_{110}$ of their initial value.

The decline of the ^{32}P of liver orthophosphate, which in view of the high permeability of liver cells corresponds closely to the fall of ^{32}P content of plasma orthophosphate, is due partly to incorporation of ^{32}P into the tissues and partly to its excretion. With increasing time excretion becomes more and more the sole way of escape of ^{32}P from the circulation. While on the first day, the ^{32}P content of orthophosphate decreases to $^1/_5$ of its 2-hour-value, a decline to $^1/_7$ of the first day's value takes 12 days, a decline to $^1/_3$ of the 12 day-value 9 days, the decrease of the 21st day value to almost $^1/_4$ takes not less than 28 days, while a decrease of half the 44 day-value requires 35 days.

The renewal of the phosphatides takes place at a slower rate than the escape of ^{32}P from the plasma, however, after a long sequence of days this difference diminishes due to the reduced loss of ^{32}P by the circulation.

The values were obtained from pooled organs of 4—6 rats weighing about 160 gm. In the 84-day experiment 0.1 millicurie was administered to each rat, in the experiments of shorter duration correspondingly less. The administration of 0.1 millicurie or more involves risks in experiments of long duration as to biological action of the radiation emitted by the administered P, as the radiation dose to which the rats are exposed during the experiment may amount to a few hundred rep.

Fat Metabolism and Phosphatide Turnover

A possible connection between fat metabolism and phosphatide turnover was repeatedly discussed. BOLLMAN and FLOCK (1946) compared the amount of phosphatides turned over in the liver and plasma with the amount of fat metabolized by the rat and arrived at the following conclusion.

Assuming, as found by these authors, 0.175 mgm of phosphatide P to be renewed per hour in a rat liver weighing 5 gm, an equal amount of phosphatide P must have been metabolized in the liver or have left this organ during that time, the latter amounting to 0.048 mgm. These figures account for a sufficient phosphatide turnover in the liver and in the plasma to metabolize or transfer fat equivalent to only 3 per cent of the caloric needs of the rat and indicate that phosphatide formation apparently is not an obligatory step in fat oxidation or transfer.

For the turnover rate of the 150 gm rat we found about twice the value given by the above authors. Thus, according to our results, the amount of phosphatides turned over should be twice the above figure and, if glycerophosphate is assumed to be the ultimate phosphatide precursor, even three times the last mentioned value, but still only 18 per cent of the caloric needs of the rat. Consequently, our findings do not contradict BOLLMAN and FLOCK's conclusion.

In the above considerátion no account was taken of the possibility of the existence of a minor phosphatide fraction which may be renewed very rapidly, as discussed on p. 361, nor was the phosphatide turnover in other organs than the liver accounted for.

Summary

(1) The extent of incorporation of intracellular orthophosphate P of the liver phosphatides of the rat decreases with increasing age. While it amounts to 12 per cent per hour for the liver phosphatides of a 4-day-old rat, the corresponding figure for a 1.5-year-old rat is 6, intermediate figures being obtained for rats of intermediate age.

(2) The calculation of the "rate of turnover" from the ratio of the end value of the specific activities of phosphatide P and the mean value of the specific activities of orthophosphate P during the experiment supplies, in experiments lasting 4 hours or less, almost identical figures with those obtained when, according to ZILVERSMIT *et al.*, the calculation is based on the change of the specific activity of phosphatide P with time.

(3) Replacement of the specific activities of liver inorganic P by corresponding values of ATP $P_{2,3}$ of the liver leads to very similar turnover rate values.

(4) The ratio of the turnover rate of the phosphatides of the total liver tissue, the mitochondria, and the cell nuclei of the liver is found to be 1 : 0.67 : 0.42.

(5) The percentage of labelled P administered present in 1 mgm liver phosphatide P of the rat, which is as high as 0.121 after the lapse of 1 day, declines to 0.00066 after the lapse of 84 days.

(6) The lower limit of the turnover of kidney phosphatides is not markedly dependent on the age of the rat and amounts to about 5 per cent in the course of 1 hour. Lower and with the age of the rat decreasing values are obtained for the spleen phosphatides. The percentage rate of incorporation of orthophosphate P per hour for a three-month-old rat can be estimated to be about 4.

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Comment on papers 34—35

We calculated in paper 29 and the following papers the rate of renewal of phosphatides from the specific activity of the phosphatide phosphorus at the end of the experiment, and of the mean specific activity of the cellular inorganic phosphorus during the experiment. The latter is obtained from the specific activities of the total inorganic P after correcting for the share of the extracellular phosphorus in the total inorganic P activity. The possibility was considered in these studies that it is not the cellular but the extracellular ^{32}P which participates in the synthesis of the organic phosphorus compounds present in the tissues and that "It is conceivable that some of the phosphatide molecules are renewed inside the cell wall." Correspondingly, all turnover data were calculated, assuming once participation of cellular ^{32}P and than of extracellular ^{32}P (paper 34). That phosphate enters the cells, at least partly, by the formation of ATP and other intermediates of the cycle on the cell membrane and that inorganic phosphate within the cell arose from the dephosphorylating reactions of the cycle was shown later by Sacks (1951).

In paper 36 in which the calculation of the turnover rate is discussed we find the following remark: "... If the incorporation of the phosphate radical into the phosphatide molecules would be preceded by the formation of glycerophosphate and this process would be a comparatively slow one, in contrast to all other steps involved in the synthesis of the phosphatide molecule, in this case the turnover rate measured, using labelled phosphorus as an indicator, would be slower than found when using labelled fatty acids or labelled choline ..." and that "the question if and to what extent the rate of labelled phosphate incorporation into the phosphatide differs, for example from that of the fatty acid incorporation into the latter cannot be answered at the time being". The question thus raised was answered by Chaikoff, Zilversmit, and their associates (1941) who demonstrated that glycerophosphate is the pertinent precursor of phosphatide synthesis and that the calculation of the turnover rate of phosphatides from the specific activity of inorganic phosphorus leads to too low a value for the turnover rate. In experiments with rats, taking 2 hr, the mean specific activity of the orthophosphate phosphorus of the liver was found to be three times that of the correponding value of glycerophosphate phosphorus (paper 35); thus, the turnover rate calculated assuming the liver orthophosphate P to be the phosphatide precursor has to be multiplied by three to arrive at a correct turnover rate value. The turnover rate of phosphatides present in the different sub-units of the liver cell was found to differ markedly (paper 35 and Hevesy, 1947). Applying palmitic acid — 1 — ^{14}C as a precursor Chaikoff *et al.* could also demonstrate that the liver is the principal site for the formation of fatty acid ester bonds of plasma phosphatide molecules.

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Originally communicated in *Nature* **141,** 1097 (1938)

36. MOLECULAR REJUVENATION OF MUSCLE TISSUE

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The decomposition of creatine phosphoric acid during muscular action and its rebuilding during rest, has been the subject of numerous detailed investigations. We were interested in the problem, if, and to what extent, creatine phosphoric acid molecules are decomposed and afterwards rebuilt, or 'rejuvenated', in the resting muscle. This problem can be easily solved by injecting labelled sodium phosphate, for example, into frogs, and determining if, and to what extent, creatine phosphoric acid extracted from the muscle of the frog becomes labelled (radioactive). Phosphorus atoms present in creatine phosphoric acid and other organic compounds do not exchange spontaneously with other phosphorus atoms present, and thus the fact that labelled creatine phosphoric acid can be isolated from the muscle is a proof that this was synthesized after the administration of labelled sodium phosphate.

The muscle was placed at once after removal in liquid air, the acid soluble components extracted with trichloracetic acid kept at $-9°$, and the inorganic phosphate present in the solution precipitated as ammonium magnesium salt. The next step was the decomposition of creatine phosphoric acid remaining in the filtrate from the last-mentioned precipitate. The decomposition was carried out by adding[1] sulphuric acid ($1n$) and ammonium molybdate (1 per cent) to the solution. The phosphate ions were then precipitated as ammonium magnesium salt. The phosphorus content of the latter was determined by the colorimetric method of Fiske and Subbarow, and its radioactivity by making use of a Geiger counter. The results obtained for this and some other fractions are seen in the accompanying table.

A specific activity of the creatine phosphoric acid phosphorus amounting to 49 per cent of that of the inorganic phosphorus indicates that 49 per cent of the creatine phosphoric acid molecules present in the resting muscle were split and newly synthesized through enzymatic action in the course of the last 3 hours before the frog was killed. As the total number of creatine phosphoric acid molecules present in the muscle can be assumed not to have changed during that time, in the resting

muscle we are faced with a molecular rejuvenation of the creatine phosphoric acid to the above extent. The adenosin and the hexosephosphate molecules are rejuvenated to about the same extent as those of the creatine phosphoric acid. With increasing temperature, as is to be expected, the rate of molecular rejuvenation increases, and in the course of less than a day practically all creatine phosphoric acid molecules are renewed.

PHOSPHORUS ISOLATED FROM FROG KILLED 3 HOURS AFTER SUBCUTANEOUS INJECTION OF LABELLED SODIUM PHOSPHATE

	Relative specific activity (activity per mgm P)	
	Frog kept at 2°	Frog kept at 21°
Inorganic P	100	100
Creatine P	49	78
Adenosin P (7 min hydrolysed at 100°)	50	—
"Hexose" P (30 min hydrolysed at 100°)	52	78
Product of 100 min hydrolysis at 100°	45	—
Non-acid soluble residual fraction	9	38

The new formation of some of the 'acid soluble' phosphorus compounds present in the blood also takes place to a very appreciable extent. In human blood 2 hours after intravenous injection of labelled sodium phosphate, the specific activity of the total acid soluble organic phosphorus, kindly extracted by Mr. A. H. W. ATEN from the blood corpuscles, amounted to 20 per cent of that of the plasma inorganic phosphorus.

In experiments *in vitro*[2] in which dog's blood was shaken for 2.5 hours with labelled sodium phosphate, 1/25 of the total acid soluble molecules was found to be labelled and thus split and resynthesized under the action of enzymes. In the same *in vitro* experiments the formation of only very minute amounts of labelled phosphatides (less than 0.1 per cent) could be ascertained. Also in experiments *in vivo* labelled phosphatides were found to be present to an appreciable extent in the blood only after much longer time. The specific activity of phosphatide P extracted from human blood corpuscles 24 hours after administration of labelled sodium phosphate was found to be 40 times less than that of plasma inorganic P, showing the very low rate of rejuvenation of the phosphatide molecules present in the blood.

There is thus a conspicuous difference in the rate of rejuvenation of some low molecular water soluble compounds, as for example, creatine phosphoric acid, adenosin phosphoric acid, hexosephosphate, and non-water soluble products like phosphatides, nucleoproteins and similar

compounds, present in the blood. This difference is closely connected with the fact that the first-mentioned compounds are at least partly rejuvenated through enzymatic action in the blood itself, while the latter are principally rejuvenated in the organs and carried from these in the blood stream. The measurement of the rate at which, for example, adenosintriphosphoric acid molecules are renewed can be conveniently used to determine the amount of enzymes the presence of which enables the exchange reaction to take place.

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37. RATE OF RENEWAL OF THE ACID SOLUBLE ORGANIC PHOSPHORUS COMPOUNDS IN THE ORGANS AND THE BLOOD OF THE RABBIT

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In a paper published recently in these Proceedings[1], the rate of renewal of the phosphatide molecules present in various organs of the rabbit and other animals was discussed. In the present publication, data on the rate of new formation of acid soluble phosphorus compounds are communicated. The acid soluble organic P compounds represent a great variety of chemically very different bodies: esters as, for example, hexosephosphate, nucleotide compounds as adenosintriphosphate, phosphagen, and other compounds. These compounds[2] are renewed at a comparatively fast rate in the organs in contradistinction to the phosphatides and desoxyribo nucleoproteins[3]. Furthermore, while the rate of new formation of the phosphatides in the circulation is almost negligible, the acid soluble phosphorus compounds are renewed at a remarkable rate in the corpuscles. These facts justify the consideration of the acid soluble phosphorus compounds from our view-point as a definite group of the phosphorus compounds present in the body.

EXPERIMENTAL METHOD

Labelled P as sodium phosphate was administered by intravenous or subcutaneous injection to rabbits all through the experiment in order to keep the activity of the plasma inorganic P at a constant level. After the lapse of some hours or days, the animal was killed by bleeding. The fresh organs were placed in liquid air and were extracted immediately with cold 10 per cent trichloroacetic acid. The inorganic phosphate

[1] G. Hevesy and L. Hahn, *Kgl. Danske Vidensk. Selskab. Biol. Medd.* **15**, 5 (1940).

[2] With the exception of adenylic acid [T. Korzybski and J. K. Parnas, *Z. physiol. Chem.* **255**, 195 (1938)] and, possibly, of other not yet known minor components of the acid soluble P mixture.

[3] L. Hahn and G. Hevesy, *Nature*, April 6, 1940.

present in the extract was precipitated as ammonium magnesium phosphate. The filtrate obtained was then hydrolysed with 1 n H_2SO_4 for 7 min at 100° to split off the labile P which was then precipitated as ammonium magnesium salt. The filtrate obtained after the last mentioned operation was hydrolysed 100 min to split off the phosphate radical of the hexosephosphate present. In order to avoid several consecutive precipitations of ammonium magnesium phosphate which lead to an accumulation of very appreciable amounts of ammonium salt in the soluble fraction, we usually divided the filtrate obtained after precipitation of the inorganic phosphate present as such in the tissue into aliquot parts. One aliquot part was hydrolysed for 7 min, the phosphate split off was precipitated, and the filtrate obtained was hydrolysed for 100 min. Another aliquot part was hydrolysed for 7 min, the filtrate obtained was hydrolysed for 12 hours, and so on. The phosphate of the creatinephosphoric acid was split off by heating the solution for 30 min to 40°. In some cases, the total acid soluble organic P was converted into phosphate and was investigated in toto. The ammonium magnesium phosphate precipitates obtained were dissolved in diluted hydrochloric acid and an aliquot part was used for a colorimetric P determination. To another aliquot part about 80 mgm non-active sodium phosphate was added; the total P present in the solution was then precipitated as ammonium magnesium salt. The radioactivity of these precipitates was determined by the aid of a GEIGER counter.

Though the separation of the different acid soluble P compounds described above is far from being quantitative, it sufficed in most cases for our purpose.

In the experiments with blood, as anti-coagulent, ammonium oxalate was used. The corpuscles were centrifuged off and washed twice with a physiological sodium chloride solution. In experiments *in vitro*, the blood was kept in a CO_2—O_2 atmosphere and was shaken, after addition of labelled sodium phosphate of negligible weight, for 30—190 min in a thermostat at 37°.

RATE OF NEW-FORMATION

As labelled phosphorus atoms can only be incorporated into organic molecules in the course of a synthetic process, the radioactivity of the organic phosphorus compounds isolated from an organ is a measure of the rate of its total or partial resynthesis. It is, however, not permitted to compare the specific activity (activity per mgm P) of the hexosemonophosphate extracted from the kidney and the muscle, for example, and to conclude from the fact that the hexosemonophosphate extracted from the kidney is much more active than that secured from the muscle,

that the rate of new formation[1] of hexosemonophosphate is correspondingly larger in the kidney. The incorporation of labelled P atoms into the hexosemonophosphate molecules must be preceded by a penetration of the labelled inorganic P into the cells of the organ. If this process is slow, the rate of formation of labelled hexosemonophosphate molecules is bound to be comparatively slow, in spite of a possibly very fast rate of new formation of the hexosemonophosphate molecules inside the cells of the organ in question. In fact, the labelled inorganic phosphate molecules penetrate very much faster into the kidney cells than into the muscle cells. To get proper information on the rate of renewal of an organic compound in an organ, we have to compare the specific activity of the P isolated from the organic compound in question at the end of the experiment with the average value of the specific activity of the cellular inorganic P prevailing during the experiment. The

TABLE 1. — EXTENT OF RENEWAL OF THE TOTAL ORGANIC ACID SOLUBLE P IN THE ORGANS OF THE RABBIT

Rabbit II. Weight 2.6 kgm.
Intravenous injection during 215 min

Organ	A Specific activity of the tissue inorganic P at the end of the experiment	B Specific activity of the cellular inorganic P at the end of the experiment	C Average specific activity of the cellular P during the experiment	D Specific activity of the organic P at the end of the experiment	$\frac{D}{C} \times 100$ Upper limit of the percentage renewed	$\frac{D}{B} \times 100$ Lower limit of the percentage renewed
Plasma	100	—	—	—	—	—
Corpuscles	12.7	12.7	6.4	12.7	199	100
Kidney	87.4	87.1	77.8	33.6	43.2	38.6
Small intestine, mucosa	47.4	45.2	22.6	24.0	106	53.1
Liver	44.0	40.6	20.4	14.3	70.2	35.2
Lungs	36.5	26.9	13.4	9.5	71.0	35.3
Spleen	30.8	28.5	14.3	—	—	—
Stomach	25.9	23.6	11.8	6.9	58.5	29.2
Heart	25.5[1]	21.4	10.8	8.6	79.6	40.2
Brain	1.32	—	—	0.56	—	—

[1] The inorganic P extracted from the heart contains partly such inorganic P atoms which were formed through decomposition of creatinephosphoric acid prior to the extraction. As the specific activity of the creatine P is, after the lapse of 4 hours, lower than that of the inorganic P (comp. the muscle values in Table 3!), the specific activity of the cellular inorganic P of the heart is in fact higher than that stated above and, correspondingly, the values of the rate of renewal of the organic acid soluble P compounds in the heart are smaller than those stated in the last and the last but one column of Table 1.

[1] The significance of the notion of the rate of new formation is discussed in the paper by G. HEVESY and L. HAHN, *Kgl. Danske Vidensk. Selskab, Biol. Medd.* **15,** 5 (1940).

considerations mentioned above are discussed in detail in the publication cited above on the turnover rate of phosphatides. In this paper is described the method which permits us to calculate from the specific activity of the tissue inorganic P. the specific activity of the plasma inorganic P, the extracellular space of the organ, and the inorganic P content of the

TABLE 2. — EXTENT OF RENEWAL OF DIFFERENT FRACTIONS OF THE ORGANIC ACID SOLUBLE P

Rabbits II, III, and IV (average)
Intravenous injection during 4 hours

Organ	Time of hydrolysis in 1 N H_2SO_4 at 100°	Spec. activity of the organic P at the end of the experiment: Average specific activity of the cellular inorganic P during the experiment. (Upper limit of the percentage renewed)	Spec. activity of the organic P at the end of the experiment: Specific activity of the cellular inorganic P at the end of the experiment. (Lower limit of the percentage renewed)
Liver	0—7 min	152	76
Liver	non-hydrolysed	66	33
Kidney, cortex	0—100 min	64	57
Kidney, cortex	100 min—12 hr.	47	42
Kidney, cortex	non-hydrolysed	29	26
Kidney, cortex	hydrolysed in 1 N NaOH at 80°	48	43

TABLE 3. — SPECIFIC ACTIVITY OF ACID SOLUBLE P FRACTIONS EXTRACTED FROM THE ORGANS OF THE RABBIT

Rabbit VII. Weight 2.4 kgm
Subcutaneous injection during 11.5 hours

Fraction	Specific activity at the end of the experiment
Plasma inorganic P	100
Corpuscle inorganic P	25
Corpuscle P hydrolysed 15 hours in 1 N H_2SO_4 at 100°	25
Corpuscle P hydrolysed 15—120 hours in 1 N H_2SO_4 at 10°	25
Corpuscle non-hydrolysed residue	13.0
Muscle inorganic P	15.5
Muscle creatine P	8.5
Marrow inorganic P[1]	13.1
Marrow organic P	36.8
Brain inorganic P	3.0
Brain organic P	2.3

[1] The low value is presumably due to the presence of traces of slightly active bone P in the marrow sample.

organ and plasma the average specific activity of the cellular inorganic P during the experiment. From the latter magnitude and the specific activity of the P of the organic phosphorus fraction at the end of the experiment, we can calculate what percentage of the organic compound in question is newly formed during the experiment, if only the extent of new formation is restricted.

If a large fraction of the hexosemonophosphate molecules, for example, is newly formed during the experiment, we can no longer disregard the number of hexosemonophosphate molecules which were decomposed and resynthesized more than once during the experiment. If such a repeated new-formation takes place, it will have the effect that the active hexosemonophosphate molecules present at the end of the experiment cannot be longer considered as having been formed with participation of inorganic P which had an activity corresponding to the average activity prevailing during the experiment. The inorganic P atoms, which had an activity corresponding to a late stage of the experiment, will

TABLE 4. — SPECIFIC ACTIVITY OF ACID SOLUBLE P FRACTIONS EXTRACTED FROM THE ORGANS OF THE RABBIT

Rabbit VIII. Weight 2.0 kgm
Subcutaneous injection during 9 days

Fraction	Specific activity at the end of the experiment
Plasma inorganic P	100
Corpuscle total acid soluble P	94
Muscle inorganic + creatine P	40
Muscle ester P	18.7
Brain inorganic + creatine P	18.8
Brain ester P	17.3

TABLE 5. — SPECIFIC ACTIVITY OF ACID SOLUBLE P FRACTIONS EXTRACTED FROM THE ORGANS OF THE RABBIT

Rabbit IX. Weight 2.5 kgm
Subcutaneous injection during 50 days

Fraction	Specific activity at the end of the experiment
Plasma inorganic P	100
Corpuscle total acid soluble P	100
Muscle inorganic + creatine P	88
Muscle ester P	77
Brain inorganic + creatine P	56
Brain ester P	68

clearly be found to a larger extent incorporated in hexosemonophosphate molecules than those P atoms the activity of which corresponds to an early stage of the experiment.

Let us consider active hexosemonophosphate molecules which were formed during the first stage of the experiment and which were again newly formed during the last minute of the experiment. If the second

TABLE 6. — SPECIFIC ACTIVITY OF ACID SOLUBLE P FRACTIONS EXTRACTED FROM THE CORPUSCLES OF THE RABBIT

Rabbits II, III, and IV (average)
Intravenous injection during 4 hours

Time of hydrolysis in 1 n H_2SO_4 at 100°	Specific activity at the end of the experiment
Inorganic P (present as such in the corpuscles)	100
0—7 min	100
7—100 min	100
7 min—12 hours	100
Non-hydrolysed in the course of 12 hours (residue)	87
Non-hydrolysed in the course of 24 hours (residue)	77

process were not forthcoming, we should find molecules of small activity; if the opposite were the case, we should find the molecules to be strongly active. When calculating the fraction of the hexosemonophosphate molecules which were newly formed (once or several times) during the experiment from the ratio

$$R = \frac{\text{specific activity of hexosemonophosphate P at the end of the experiment}}{\text{average specific activity of inorganic P during the experiment}}$$

we overestimate the percentage of hexosemonophosphate which was renewed during the experiment. This will be especially the case if the ratio

$$\frac{\text{rate of renewal}}{\text{rate of intrusion}}$$

is large, as, for example, in the case of the corpuscles.

When calculating from the ratio

$$\frac{\text{specific activity of organic P at the end of the experiment}}{\text{average specific activity of inorganic P during the experiment}}$$

the extent of renewal of the acid soluble P mixture in the corpuscles, we arrive at a value of 199 per cent (see Table I). Such a calculation, for reasons stated above, supplies the upper limit of the extent of renewal. The lower limit is given by the ratio:

$$\frac{\text{specific activity of organic P at the end of the experiment}}{\text{specific activity of inorganic P at the end of the experiment}}.$$

The actual value clearly lies very much nearer to the lower than to the upper limit.

TABLE 7. — EXPERIMENTS *in vitro* WITH RABBIT BLOOD

Corpuscle fraction	Duration of the experiment	Specific activity at the end of the experiment
Hydrolysed 7 min	30 „	77
Hydrolysed 7 min—12 hours	30 „	16
Non-hydrolysed	30 „	13
Inorganic P	60 „	100
Hydrolysed 7 min	60 „	90
Hydrolysed 7 min—12 hours	60 „	41
Non-hydrolysed	60 „	28
Inorganic P	90 „	100
Hydrolysed 7 min	90 „	82
Hydrolysed 7 min—12 hours	90 „	57
Non-hydrolysed	90 „	46
Inorganic P	190 „	100
Organic acid soluble P	190 „	57

TABLE 8a. — EFFECT OF TEMPERATURE ON THE DISTRIBUTION OF ^{32}P BETWEEN PLASMA AND CORPUSCLES

Rabbits blood after addition of labelled phosphate of negligible weight is shaken for 90 min

Fraction	Temperature	
	37°	5°
Plasma inorganic P	78	9.67
Corpuscle inorganic P	3.8	0.62
Corpuscle organic P	18.2	2.64

TABLE 8b. — EFFECT OF TEMPERATURE ON THE DISTRIBUTION OF ^{32}P BETWEEN PLASMA AND CORPUSCLES

Relative specific activity of the P fractions of the blood

Fraction	Temperature	
	7°	5°
Plasma inorganic P	100	100
Corpuscle inorganic P	13.8	0.37
Corpuscle pyrophosphate P	11.3	0.36
Corpuscle non-hydrolysed P	6.9	0.087

Table 8c. — Effect of Temperature on the ^{32}P Fractions of the Corpuscles

Relative specific activity of the P fractions of the corpuscles

Fraction	Temperature	
	37°	5°
Corpuscle inorganic P	100	100
Corpuscle pyrophosphate P	82	84
Corpuscle non-hydrolysed P	50	23.5

DISCUSSION

A. Renewal of the acid soluble P compounds in the organs.
B. Renewal of the acid soluble P compounds in the corpuscles.

A) Renewal of the acid soluble P compounds present in the organs

As seen in Table 1, in the course of 4 hours a very appreciable part of the average acid soluble P compounds present in many of the organs was renewed. A very active turnover takes place in the mucosa of the small intestine. One half or more of the molecules of the organic acid soluble P compounds present in this organ became renewed in the course of 215 min. This very marked rate of new formation of the organic acid soluble P compounds is of interest in connection with the view put forward by Verzár and others on the role of intermediary phosphorylation processes in the resorption of sugar from the intestine[1]. The highest value for the specific activity of the acid soluble organic P was found in the kidneys. The labelled inorganic P diffuses faster into the cells of the kidneys than into those of any other organ. The high value of the specific activity of the acid soluble kidney P is, to some extent, due to the fact that the cellular inorganic P within 215 min acquires a higher value in the kidneys than in other organs. If due regard is taken to this phenomenon we find that, in spite of the fact that the specific activity of the intestinal acid soluble P is lower than that of the corresponding fraction extracted from the kidneys, the rate of renewal in the intestinal mucosa is greater than in the kidneys.

The rate of renewal of the organic acid soluble P molecules in the liver and in the lungs (see Table 1) is also quite appreciable. The comparatively high value found for the ratio of the specific activities of the organic P and inorganic P in the case of the brain tissue is, at least to

[1] F. Verzár and E. J. McDougall, *Absorption from the Intestine*. London (1936). Comp. also E. Lundsgaard, *Z. physiol. Chem.* **261**, 19 (1939).

some extent, due to an extremely low activity of the average inorganic P of the brain. It is a puzzling result that the total activity found in the brain tissue, due to the presence of active inorganic and organic P, is smaller than that we should expect to find in the interspaces of the brain alone when assuming a proportional distribution of the active inorganic P between the plasma and the extracellular space of the brain tissue. In this calculation, the extracellular space is taken to be 30 per cent of the weight of the brain, as found from the distribution figures of chlorine and sodium[1] between the plasma and the brain tissue. Our results suggest the assumption that the labelled phosphate ions penetrate at a very slow rate through the capillaries of the brain or, alternatively, that the figures obtained by determining the distribution of chlorine of sodium between the plasma and the brain do not represent the proper extracellular space of the brain. It is for these reasons that we did not state in Table 1 any figures for the rate of renewal of the acid soluble P compounds present in the brain.

Table 2 contains data on the activity of different organic P fractions extracted from the kidneys and the liver. The phosphate obtained after 7 min hydrolysis contains, as well-known, besides P split off from creatinephosphoric acid, the labile P of the adenosintriphosphate molecules. That the adenosintriphosphate molecules present in the muscle are reorganised at a fast rate was found in our previous experiments[2] MEYERHOF and his collaborators[3] studied the rate of reorganistion of the adenosintriphosphate molecule with incorporation of active inorganic P in experiments *in vitro* and found this process to take place at a very fast rate. Data on the activity of the phosphorus obtained by hydrolysing the organic acid soluble phosphorus extracted from perfused cat liver for 7 min are given by LUNDSGAARD[4].

Our experiments lead to the result that at least 76 per cent of the 7 min product extracted from the liver of the rabbit became renewed in the course of 215 min. In LUNDSGAARD's perfusion experiment, the specific activity of the 7 min fraction was found, after 90 min, to amount to 60 per cent of that of the inorganic P extracted from the plasma at the end of the experiment.

As seen in Tables 2—7 the more readily hydrolysable compound is renewed at a faster rate than the less readily hydrolysable one. That even those compounds which resist treatment with 1 N H_2SO_4 at 100° for 12 hours or more are renewed, however, at a very appreciable rate

[1] J. F. MANERY and B. HASTINGS, *J. Biol. Chem.* **127,** 657 (1939).

[2] G. HEVESY and O. REBBE, *Nature* **141,** 1097 (1938); G. HEVESY, *Enzymologia* **5,** 138 (1938).

[3] O. MEYERHOF, P. OHLMEYER, W. GENTNER and H. MAIER-LEIBNITZ, *Biochem. Z.* **298,** 398 (1938).

[4] E. LUNDSGAARD, *Skand. Arch. f. Physiol.* **80,** 291 (1938).

is seen in Tables 2 and 3. More than 1/4 of the non-hydrolysable residue of the organic acid soluble P fraction secured from the kidneys was, for example, found to be renewed in the course of 215 min (see Table 2).

After the lapse of so long a time as 9 and 50 days (see Tables 4 and 5), the muscle inorganic + creatine P has only reached 40 and 88 per cent, respectively, of the specific activity of the plasma inorganic P. After the lapse of 50 days, the specific activity of the ester P of the muscles was found to be 77 per cent of that of the plasma inorganic P. A detailed investigation of the rate of renewal of the acid soluble P compounds present in the muscles of the frog will be published shortly.

B) Renewal of the acid soluble P compounds present in the corpuscles

1. Phosphorylation processes going on inside the corpuscles

In our early investigations[1] on the circulation of phosphorus, using radioactive P as an indicator, we found that the organic acid soluble P compounds of the red blood corpuscles are normally in a state of flux, being continuously decomposed and resynthesized. Labelled phosphate ions were found to penetrate into the corpuscles at a fairly slow rate and to take part in very rapid phosphorylation processes inside the corpuscles. Labelled hexosemonophosphate introduced into the plasma was found not to penetrate at any significant rate into the corpuscles. However, the labelled phosphate present in such hexosemonophosphate molecules after being split off diffuses as inorganic phosphate into the corpuscles and is incorporated inside the erythrocytes partly into hexosemonophosphate molecules. Presumably, the P atoms of the plasma diffuse exclusively or almost exclusively as phosphate ions into the corpuscles.

That phosphorus compounds, as hexosephosphoric acid, triosephosphoric acid, phosphopyruvic acid, phosphoglyceric acid, and so on, take an important part in glycolytic processes going on in the corpuscles was emphasised by v. Euler and Brandt[2], and others. According to the views of Meyerhof, Parnas, and others, in the course of the glycolytic cycle, hexosediphosphate, for example, is found to be formed through the interaction of dextrose with adenosintriphosphate. Hexosediphosphate is maintained in enzymatic equilibrium with two molecules

[1] L. Hahn and G. Hevesy, *C. R. Lab. Carlsberg* **22,** 188 (1938). G. Hevesy and A. H. W. Aten, *Kgl. Danske Vidensk. Selskab, Biol. Medd.* **14,** 5 (1939).

[2] H. v. Euler and K. M. Brandt, *T. physiol. Chem.* **240,** 215 (1936). Comp. also H. Lawaczeck, *Biochem. Z.* **145,** 351 (1924); Negelein, *Biochem. Z.* **158,** 121 (1925); M. Martland, *Biochem. J.* **19,** 117 (1925); P. Rona and K. Iwasaki, *Biochem. Z.* **184,** 318 (1917); H. K. Barrenscheen and B. Vásárhelyi, *Biochem. Z.* **230,** 330 (1931); H. K. Barrenscheen and K. Braun, *Biochem. Z.* **231,** 144 (1931).

of triosephophate. The last mentioned compound reacting with pyruvic acid forms phosphoglyceric acid which is converted into phosphopyruvic acid and this, in turn, reacts with adenylic acid in the resynthesis of adenosintriphosphate. The last mentioned compound is also formed by direct phosphorylation of adenylic acid from inorganic phosphate or by transfer of the phosphate radical of glycerophosphate to adenylic acid. The synthesis of adenosintriphosphate is a very rapid process and the active inorganic phosphate ions which penetrate into the corpuscles will soon be found to be incorporated in adenosintriphosphate molecules. The participation of the active adenosintriphosphate molecules in the synthesis of various organic P compounds will lead to the formation of active hexosephosphate, active phosphoglyceric acid, and so on, in the corpuscles. In this connection, the result obtained by DISCHE[1] is of interest: he found that the total phosphate transferred to glucose added to human erythrocytes originates from adenosintriphosphate.

Important evidence that the organic acid soluble phosphorus compounds and, primarily, diphosphoglycerate of the red blood corpuscles constitute a labile phosphorus reserve of considerable consequence, serving various functions was presented in recent years by GUEST and his colleagues[2]. Some of their findings are described in what follows.

The development of rickets in rats is associated with decreases in all fractions of the acid soluble phosphorus. During the first five days, the concentration of inorganic phosphorus and adenosintriphosphate phosphorus drops abruptly to a low level and then remains constant for 25 days and longer. The decrease in the organic acid soluble phosphorus is accounted for almost entirely, after the first few days, in the diphosphoglycerate fraction. GUEST and RAPPAPORT state that diphosphoglycerate makes out about half of the acid soluble phosphorus present in the corpuscles.

In experiments carried out on dogs after nephrectomy, it was found that, due to the failure of excretion of the vaste endogenous P, a large increase in the inorganic P content of the blood takes place, which is followed by a corresponding increase in the acid soluble organic P content of the corpuscles. The increase is mainly due to the rise of the diphosphoglycerate content of the corpuscles, the increase in organic acid soluble P and in diphosphoglycerate P being 47 and 43 mgm. respectively, per hundred cc.

They found, furthermore, that the increase of phosphorus excretion in the urine during acidosis comes partly from mobilised diphospho-

[1] Z. DISCHE, *Naturwiss.* **24,** 462 (1936).

[2] A summary of many of their results is to be found in the paper by G. M. GUEST and S. RAPPAPORT, *Amer. J. Dis. Children* **58,** 1072 (1939).

glycerate of the corpuscles. As an effect of pyloric obstruction, an increase of the acid soluble P content amounting to 37 mgm. equiv. per kgm corpuscle water of the dog was found to take place. From this increase, 32 mgm equiv. were due to the rise in the glycerophosphate content.

These and numerous other findings clearly show that the acid soluble phosphorus compounds of the red corpuscles are readily synthesised and decomposed in the blood through reactions of the glycolytic cycle. That these processes take place in the corpuscles at a remarkable speed was shown by us when making use of radioactive phosphorus as an indicator. We have, thus, two independent lines of evidence as to the remarkably high rate of turnover of phosphoglycerate and some other phosphorus compounds present in the corpuscles.

By comparing the specific activity of the inorganic P of the corpuscles with that of the P extracted from various organic compounds present in the corpuscles we get information on the rate of resynthesis of these compounds. The comparison of the specific activity of the inorganic P present in the corpuscles with that of the inorganic P present in the plasma informs us, on the other hand, on the rate of penetration of phosphate ions from the plasma into the corpuscles.

2. Rate of new formation of the acid soluble P compounds present in the corpuscles

As seen in Table 7, which gives the result of experiments *in vitro*, the product of 7 min hydrolysis has, after the lapse of 30 min, a specific activity amounting to 77 per cent of that of the corpuscle, inorganic P. The product hydrolysed between 7 min and 12 hours, which contains besides hexosephosphate P and other fractions appreciable amounts of diphosphoglycerate P as well, is markedly less active than the readily hydrolysed fraction, while the specific activity of the P of the non-hydrolysed residue is only 1/8 of that of the corpuscle inorganic P. This fraction[1] consists mainly of 2, 3-diphosphoglyceric acid P though it contains also P of the adenylic acid which amounts, in the corpuscles of the rabbit, to about 5—10 mgm per cent, thus to about 1/10—1/20 of the total acid soluble P of the corpuscles.

In experiments *in vivo* taking about four hours, all but the non-hydrolysed fraction were found to be entirely renewed; only about 1/5 of the last mentioned fraction, presumably mainly its adenylic acid

[1] E. Greenwald, *J. Biol. Chem.* **63,** 339 (1925); H. Jost, *Z. physiol. Chem.* **116,** 171 (1927); S. E. Kerr and A. Autaki, *J. Biol. Chem.* **121,** 531 (1927); E. Warweg and G. Stearns, *J. Biol. Chem.* **115,** 567 (1936); S. Rappaport and G. M. Guest, *J. Biol. Chem.* **129,** 781 (1939); A. Lennerstrand and M. Lennerstrand, *Ark. Kemi,* **13,** B, No. 15 (1939).

content[1], was found to be unchanged. Diphosphoglyceric acid is, thus, renewed at a high rate as well.

Rate of penetration of plasma inorganic P into the corpuscles

To obtain information on the rate of penetration of the inorganic phosphate of the plasma into the corpuscles, we have to compare the specific activity of the plasma inorganic P with that of the corpuscle inorganic P. After the lapse of 11.5 hours (see Table 3), this ratio is found to be 4, showing that the rate of penetration of the phosphate ions from the plasma into the corpuscles and vice versa is slow, a much slower process than the reorganisation of most of the acid soluble organic P compounds present in the corpuscles. After the lapse of nine days, the ratio of the specific activity of the plasma inorganic P and the corpuscle average acid soluble P is only slightly larger than 1 (1.06) (after so long a time, the activity of the average corpuscle acid soluble P acquired almost the same value as shown by the inorganic P of the corpuscles); and after the lapse of fifty days, a completely proportional distribution of the labelled P atoms between the plasma P and the P of the acid soluble P compounds present in the corpuscles is attained. While, after the lapse of 11.5 hours, the chance of a normal distribution of a P atom which diffused into the corpuscles between organic and inorganic P is almost 1, the corresponding figure for the distribution of an inorganic P atom between plasma and corpuscles is only of the order of magnitude of 1/4.

The interesting phenomenon that an individual phosphate ion, while penetrating fairly slowly into the corpuscles, is incorporated at a remarkably fast rate into organic molecules present in the corpuscles, finds many analoga in the processes going on in various organs. It is especially conspicuously shown in the study of the penetration of labelled phosphate into the muscle cells and of that of the rate of renewal of the acid soluble P compounds present in these cells; the former process being slow, the latter process being, in the case of some of the compounds, very fast.

It is very probable that a large part of the P atoms present in the molecules, of most of the acid soluble organic P compounds of the corpuscles, were incorporated into these molecules inside the corpuscles and reached the erythrocytes as inorganic phosphate ions which passed from the plasma into the corpuscles. The possibility that hand in hand with the process mentioned above a slow exchange of, for example, organic phosphoglycerate between plasma and corpuscles

[1] S. E. Kerr and L. Daoud [*J. Biol. Chem.* **109**, 304 (1937)] state that, out of 88 mgm per cent organic acid soluble P found in the corpuscles of the rabbit, 16 mgm per cent are pyrophosphate P and 8 mgm per cent adenylic acid P.

takes place cannot be disregarded. In view of the very low content of organic acid soluble P compounds of the plasma, if a migration of such compounds between corpuscles and plasma would take place, it should be mainly directed from the corpuscles into the plasma. In view of the fast rate of renewal going on in the corpuscles and the fast turnover of the acid soluble P compounds in the plasma, the investigation of a migration of organic acid soluble P molecules from the corpuscles into the plasma or vice versa encounters difficulties.

In the above connection it is of interest to remark that SOLOMON, HALD and PETERS[1] found, in a recent investigation, that phosphate esters present in the corpuscles are restrained from escaping by some force in addition to the membrane of the corpuscles. The restraining force is presumably a chemical aggregation or combination with substances of large molecular size. They arrived at the result mentioned above by the following observation. When filtering blood which was hemolysed by freezing, the ultrafiltrate obtained at 7° did not contain any appreciable amount of organic P, while the opposite was the case when saponin was used to obtain hemolysis. Frozen blood acts as much as does intact blood so far as phosphates are concerned. The organic esters remain intact as long as the blood is kept cold and their combination with substances of high molecular size remains unpaired. This is not the case when saponin is added. Under the action of this agency the binding forces break down, and the organic phosphate esters can enter the ultrafiltrate. At 37° the phosphate esters can be ultrafiltrated even if the blood was hemolysed by freezing. In experiments *in vitro* with intact blood at 37°, during 18 hours no appreciable amount of organic phosphate ester was found to escape from the corpuscles into the plasma. These results support the view that the P atoms present in the phosphate ester molecules of the corpuscles reach the plasma, and vice versa, after being converted into constituents of organic phosphate ions.

As seen in Tables 8 *a*, *b* and *c*, with decreasing temperature the rate of penetration of ^{32}P from the plasma into the corpuscles and also the rate of its incorporation into organic P compounds strongly decreases. While at 37°, in the course of 90 min, 22 per cent of the ^{32}P originally present in the plasma diffused into the corpuscles, at 5° only 3.3 per cent of the ^{32}P originally present in the plasma found their way into the corpuscles. The comparison of the specific activity of the inorganic P present in the corpuscles at 37° and 5°, respectively, leads to the result that this activity is 37 times larger at 37° than at 5°. A similar comparison of the specific activity of the organic P of the corpuscles (exclusive the labile P of adenosintriphosphate) leads to a ratio of 80. It is of interest to note that a decrease of the temperature hardly affects

[1] P. C. SOLOMON, P. M. HALD and J. P. PETERS, *J. Biol. Chem.* **132,** 721 (1940).

the very fast rate of new-formation of adenosintriphosphate molecules, since more than 80 per cent of the labile P of the adenosintriphosphate present in the corpuscles became renewed during the experiments both at high and at low temperatures.

Summary

Labelled phosphate was administered to rabbits all through the experiments in order to keep the activity of the inorganic phosphate of the plasma at a constant level. The experiments took 215 min to 50 days. The comparison of the specific activities of the organic P and the cellular inorganic P extracted from the organs leads to the result that in the course of 215 min more than one-half of the acid soluble P compounds present in the mucosa of the small intestine became renewed. Next the intestinal mucosa, the fastest rate of turnover was found to take place in the kidneys, liver and lungs.

From the various organic acid soluble phosphorus compounds the most readily hydrolysable ones were found to be renewed at the fastest rate. Fractions containing mainly phosphoglycerate were found to be renewed at an appreciable rate as well.

While the rate of formation of labelled acid soluble organic P compounds inside the corpuscles is rapid, the diffusion of labelled phosphate ions from the plasma into the corpuscles is a slow process. In the course of 12 hours, only about 1/4 of the P atoms of the acid soluble phosphorus compounds of the corpuscles entered into exchange equilibrium with the P atoms of the plasma phosphate, while most of the molecules of the compounds mentioned above were renewed during this time inside the erythrocytes. The contrast between the rate of interpenetration of labelled phosphate and that of its incorporation into several of the acid soluble phosphorus compounds inside the cells is also found in the case of the muscles.

Labelled phosphate was found to penetrate into the brain tissue at an exceedngly low rate.

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38. CIRCULATION OF PHOSPHORUS IN THE FROG

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This paper contains the description of some experiments on the circulation of phosphorus in the frog using radiophosphorus as an indicator. The experiments described were carried out in the course of the last six years[1].

EXPERIMENTAL PROCEDURE

Most of the radioactive phosphorus (^{32}P) used in these experiments was prepared by irradiating carbon disulphide with neutrons emitted by a radium-beryllium source. The ^{32}P produced was extracted with diluted nitric acid and turned into sodium phosphate. This sodium phosphate of negligible weight (10^{-10} gm or less) was dissolved in 0.6 per cent sodium chloride solution. A few tenths of a cubic centimetre of the solution were injected into the lymph sack of *Rana esculenta* or *Rana hungarica*. In our earlier experiments, the injection took place at the start of the experiment; in our later work, however, we injected labelled phosphate several times during the experiment in order to keep the activity of the plasma at an approximately constant level. Experiments were carried out both at 0° and at about 20°; their duration varied between 5 minutes and 400 hours. The muscle tissue was put into liquid air immediately after its removal and the acid soluble constituents were extracted with cold 10 per cent trichloroacetic acid. The filtrate obtained was at once added to a solution containing ammonia and magnesium citrate. By this procedure, all inorganic P present in the trichloroacetic extract was precipitated. The next step was to hydrolyse the creatinephosphoric acid present in the filtrate. In our early work, we decomposed the creatinephosphoric acid by adding ammonium molybdate and keeping the solution for 1 hour at 40° in 1 N. H_2SO_4. Later, we omitted the addition of ammonium molybdate and split off the phosphate by letting the acidified solution boil for a short time, as proposed by Meyerhof *et alia*.[2] The phosphate group, split off from the creatinephosphoric acid, was then precipitated as ammonium magnesium

[1] O. Rebbe, M. Sc., who has taken the deepest interest in the problems discussed in this paper and worked for several years almost incessantly on their elucidation suffered an untimely death the 5th of December, 1940.

[2] O. Meyerhof, P. Ohlmeher, W. Gentner and H. Maier-Leibnitz, *Biochem. Z.* **298**, 400 (1938).

salt. The filtrate thus obtained was acidified and the 1 N. H_2SO_4 solution obtained was boiled for 7 minutes in order to split off the pyrophosphate group of the adenosintriphosphate. In other experiments, the solution was kept at 100° for 100 minutes. By this procedure, the hexosemonophosphate was hydrolysed. Other fractions were secured by hydrolysing the filtrate for some hours. The non-hydrolysed phosphorus compounds remained in the filtrate together with large amounts of neutral salts. From this solution, after wet ashing of the organic compounds present, the phosphorus was precipitated as molybdenum compound. The ammonium phosphomolybdate was dissolved in ammonia, and phosphorus was precipitated as ammonium magnesium phosphate.

The muscle fraction which does not dissolve in trichloroacetic acid contains the phosphatides and the residual P. To secure the phosphatides, BLOOR's method was used. All fractions were ultimately obtained as ammonium magnesium phosphate which was dissolved in dilute hydrochloric acid. An aliquot part of the solution was used for the colorimetric determination of the phosphorus, according to FISKE and SUBBAROW, another aliquot part was applied in the radioactive measurements. The comparison of the radioactivity of the P fractions is much facilitated if all samples have the same weight. In this case, no correction for the absorption of the β-rays in the sample has to be made. Fractions of equal weight were obtained by adding to the solution of each fraction 80 mgm of secondary sodium phosphate and by precipitating all P present as ammonium magnesium phosphate. The precipitates obtained were dried at 106°. Corrections for the decay of the activity of the ^{32}P can also be avoided if all samples are measured relatively to the same active phosphorus preparation. As such preparation an aliquot of the solution administered was used, the P content of the preparation being converted into ammonium magnesium phosphate, as described above.

DISTRIBUTION OF PHOSPHORUS IN THE FROG

We determined the phosphorus content of the different parts of the frog by wet ashing of the organs followed by a phosphorus determination by the method of FISKE and SUBBAROW. The results of these determinations are seen in Tables 1 *a* and 1 *b*.

ABSORPTION OF PHOSPHATE

In order to get data on the rate of absorption of the labelled phosphate injected into the lymph sack, we determined the activity of plasma samples of known weight both of frogs kept at O° and frogs kept at 18° at different times after injecting the labelled phosphate. The figures obtained (see Figs. 1 and 2) are not a direct measure of the amount of ^{32}P absorbed but indicate the difference between the amount absorbed into the circulation and the amount which left the circulation for the organs. (The amount taken up by the corpuscles in the course of a few hours is very restricted; see p. 400.)

TABLE 1*a*. — DISTRIBUTION OF PHOSPHORUS IN *Rana esculenta* WEIGHING 58 GM

Organ	mgm P	Per cent of total P
Blood[1]	0.83	0.18
Skin	31.50	6.96
Muscles	59.32	13.20
Bones	335.30	74.16
Liver[2]	4.28	0.94
Remaining part	20.61	4.56
Total	451.84	

[1] Extrapolated value, assuming the blood content to constitute 4 per cent of the weight of the frog.
[2] The P content of the liver phosphatides was found to be 22 per cent of the total P content of the liver.

TABLE 1*b*. — DISTRIBUTION OF PHOSPHORUS IN THE MUSCLE OF THE FROG

Fraction	Per cent of total P content
Acid soluble P	82.1
Phosphatide P	10.8
Residual P	7.1

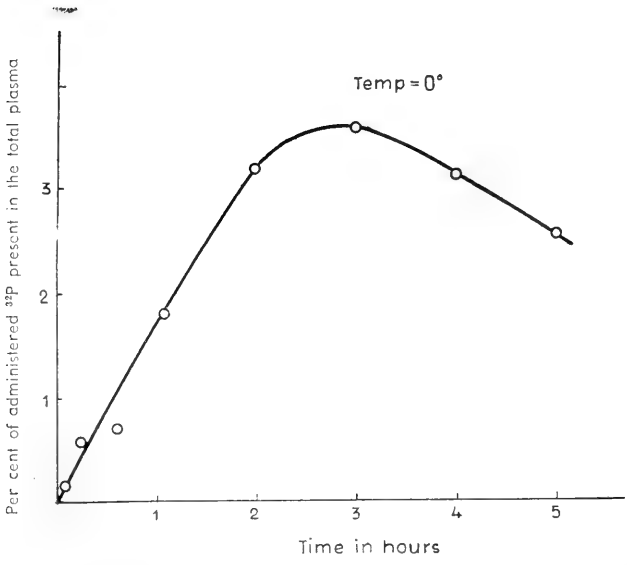

FIG. 1. ^{32}P content of the frog's plasma after injecting ^{32}P into the lymph sack. T = 0°.

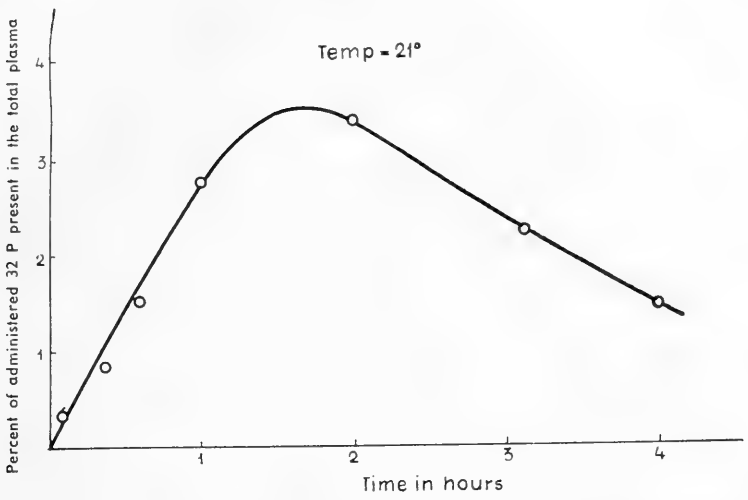

FIG. 2. ^{32}P content of the frog's plasma after injecting ^{32}P into the lymph sack. T = 21°.

In another set of experiments, we injected large amounts of phosphate (corresponding to 6.1 mgm P) into the lymph sack of the frog. The inorganic P content of the plasma at different times is seen in Table 2.

TABLE 2. — P CONTENT OF THE PLASMA AFTER INJECTING 6.1 MGM. P INTO THE LYMPH SACK OF THE FROG

Time in hours	Weight of the frog in gm	P content of the plasma mgm per cent	Excess P present in the plasma mgm per cent
0.5	76	21.7	18.1
1.5	78	23.9	20.3
19.0	50	10.3	6.7

RELATIVE RATE OF UPTAKE OF LABELLED PHOSPHORUS BY THE BONE AND THE MUSCLE

In our preliminary experiments, the results of which are reported in Tables 3—10, we compared the uptake of labelled phosphorus by fresh bone and muscle samples of equal weight at 2° and 22°, respectively. 1 gm muscle tissue was found to take up less ^{32}P than 1 gm bone tissue, though the difference is much less than would be expected if

TABLE 3. — FROG I, KEPT AT 2°, KILLED AFTER 12 DAYS

Ash weight: 3.235 gm

	Weight of fresh tissue	Weight of ash	Per cent labelled P administered found in 1 gm fresh tissue
		Bones	
	mgm	mgm	
Right femur	427.8	126.3	4.33
Left femur	442.8	123.6	4.74
Right tibia	469.5	149.2	4.76
Left tibia	432.6	141.1	5.23
		Muscles	
	gm		
Right thigh	3.989	—	0.284
Left thigh	3.467	—	0.262
Right calf	1.401	—	0.237
Left calf	1.336	—	0.199

1 mgm bone P had the same chance to be replaced by labelled P as 1 mgm muscle P. 1 gm bone tissue contains about 30 times as much P as 1 gm muscle tissue. The comparatively low activity of the bone P is due to the fact that those phosphorus ions which are located on the

TABLE 4. — FROG II, KEPT AT 2°, KILLED AFTER 12 DAYS

Ash weight: 3.014 gm

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm fresh tissue
		Bones	
	mgm	mgm	
Right femur	417.0	123.9	4.74
Left femur	425.4	116.1	4.38
Right tibia	450.6	150.8	5.01
Left tibia	450.7	152.2	4.74
		Muscles	
	gm		
Right thigh	5.461	—	0.24
Left thigh	4.635	—	0.23
Right calf	1.670	—	0.28
Left calf	1.679	—	0.31

surface of the apatite crystallites containing the mineral constituents of the bone can be replaced by a physical exchange process with the labelled phosphate in the plasma or the lymph, while the phosphate ions present inside these crystals cannot be replaced. Labelled phosphate ions can only be incorporated into the inside of the apatite crystallites

TABLE 5. — FROG III, KEPT AT 2°, KILLED AFTER 21 DAYS

Ash weight: 3.104 gm

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm fresh tissue
		Bones	
	mgm.	mgm	
Right tibia epiphysis	155.1	29.2	6.03
Left tibia epiphysis	142.9	29.1	5.90
Right tibia diaphysis	205.7	77.0	6.34
Left tibia diaphysis	200.4	76.1	6.50
		Muscles	
	gm		
Right calf	1.269	—	0.51
Left calf	1.252	—	0.42

during the formation of such crystallites from a plasma containing labelled phosphate. The dissolution of "old" apatite crystals and the formation of "new" ones is, however, a comparatively slow process and is restricted to a fraction of the bone apatite. In contrast to the

TABLE 6. — FROG IV, KEPT AT 2°, KILLED AFTER 22 DAYS

Ash weight: 3.347 gm

	Weigt of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm fresh tissue
		Bones	
	mgm	mgm	
Right tibia epiphysis	144.2	33.1	3.68
Left tibia epiphysis	158.7	39.6	4.50
Right tibia diaphysis	205.8	86.6	5.07
Left tibia diaphysis	215.2	80.8	4.52
		Muscles	
	gm		
Right thigh	4.116	—	0.26
Left thigh	4.109	—	0.29
Right calf	1.477	—	0.34
Left calf	1.548	—	0.36

bones, the greatest part of the phosphorus in the muscles is present as a constituent of organic compounds, as seen in Table 1 *b*. The bulk (about 80 per cent) of the organic P is present in the muscle of the frog in the form of acid soluble phosphorus compounds and, in a corresponding manner, the rate of activation of the muscle P depends

mainly upon the rate of formation of active acid soluble P compounds. This process is much faster than the formation of the apatite crystals of the bone tissue and this fact explains why the replacement of phosphorus in the muscle tissue takes place at a much more rapid rate

TABLE 7. — FROG V, KEPT AT 20—24°, KILLED AFTER 8 DAYS

Ash weight: 3.65 gm

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm fresh tissue
		Bones	
	mgm	mgm	
Right femur epiphysis	172.9	33.5	3.30
Left femur epiphysis	183.9	36.9	3.22
Right femur diaphysis	412.7	123.5	2.99
Left femur diaphysis	436.4	123.9	2.84
Right tibia epiphysis	265.9	59.6	2.92
Left tibia epiphysis	237.1	62.4	3.16
Right tibia diaphysis	374.4	141.9	3.02
Left tibia diaphysis	358.7	130.2	2.91
		Muscles	
	gm		
Right thigh	5.372	—	1.04
Left thigh	5.632	—	0.92
Right calf	1.925	—	1.12
Left calf	1.980	—	1.11

TABLE 8. — FROG VI, KEPT AT 22°, KILLED AFTER 8 DAYS

Ash weight: 3.46 gm

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm fresh tissue
		Bones	
	mgm	mgm	
Right femur epiphysis	150.6	37.1	4.44
Left femur epiphysis	169.8	37.6	2.66
Right femur diaphysis	398.9	130.4	3.26
Left femur diaphysis	382.0	122.3	3.20
Right tibia epiphysis	263.9	60.2	2.97
Left tibia epiphysis	252.4	60.7	3.12
Right tibia diaphysis	304.3	122.6	3.66
Left tibia diaphysis	282.5	120.9	3.42
		Muscles	
	gm		
Right calf	1.520	—	1.1
Left calf	1.584	—	1.2

TABLE 9. — FROG VII, KEPT AT 20—24°, KILLED AFTER 12 DAYS

Weight: at the start 56.5 gm; at the end 49.5 gm
Ash weight: 2.359 gm

	Weight of fresh tissue	Weight of ash	Per cent of labelled P administered found in 1 gm. fresh tissue
		Bones	
	mgm	mgm	
Right femur epiphysis	79.8	17.1	4.27
Left femur epiphysis	77.6	16.7	4.35
Right femur diaphysis	97.9	49.1	8.02
Left femur diaphysis	103.1	48.5	4.70
		Muscles	
	gm.		
Right thigh	2.608	—	1.1
Left thigh	2.139	—	1.1
Right calf	1.067	—	0.9
Left calf	1.630	—	1.1

than the replacement of phosphorus in the bone tissue. From the fact that the ratio of the uptake of ^{32}P by 1 gm muscle tissue and 1 gm bone tissue decreases much with increasing temperature (see Table 10), we can conclude that the temperature coefficient of the penetration

TABLE 10. — COMPARISON OF THE LABELLED P CONTENT OF BONES AND MUSCLES

Frog	Duration of the experiment days	Ratio of the labelled P content of bone and muscle of equal weight
	Temperature: 2°	
I	12	19.4
II	12	17.7
III	21	12.8 (epiphysis)
III	21	13.9 (diaphysis)
IV	22	13.2 (epiphysis)
IV	22	15.5 (diaphysis)
	Average value...	15.4
	Temperature: 22°	
V	8	3.0 (epiphysis)
V	8	2.9 (diaphysis)
VI	8	2.9 (epiphysis)
VI	8	2.9 (diaphysis)
VII	12	4.1 (epiphysis)
VII	12	4.6 (diaphysis)
	Average value...	3.4

and subsequent incorporation of labelled phosphate into the organic compounds of the muscle of the frog is much greater than the temperature coefficient of the formation of apatite crystals.

EXCRETION OF LABELLED P

In a few cases, we determined the percentage ^{32}P which was excreted by the kidneys of the frog. In one experiment, 1.5 cc. 0.6 per cent sodium chloride solution containing 0.008 mgm P as phosphate was injected into the lymph sack of the frog weighing 55 gm and kept at 18°. Urine was collected during 14 hours and the ^{32}P content of the urine was determined. It was found to make out 10.6 per cent of the ^{32}P injected while, in other experiments, 7.1 and 5.8 per cent, respectively, was found.

UPTAKE BY THE FROG OF ^{32}P FROM A SOLUTION CONTAINING LABELLED SODIUM PHOSPHATE

A frog weighing 88 gm was kept at 18° in 100 cc. 0.6 per cent sodium chloride solution containing 4 mgm labelled P as sodium phosphate. The solution was renewed every day. After the lapse of 2½ days, the frog was washed, killed and its P content extracted. It was found to be 695 mgm or 7.9 mgm per gram of fresh weight of the frog. The specific activity of this P was found to constitute 1/450 of the specific activity of the P of the solution in which the frog was kept. Thus, in the course of 2½ days, 1/450 of the total P of the frog was replaced by solution P. We investigated, furthermore, the activity of the inorganic P extracted from the liver of the frog which was found to show a specific activity amounting to 0.99 per cent of the specific activity of the solution P. It was, thus, 4.5 times more active than the average P of the frog.

RATE OF RENEWAL OF THE PHOSPHORUS COMPOUNDS IN THE MUSCLE

In the preceding sections, experiments were described in which the percentage of the administered ^{32}P present in the skeleton and the muscles was determined. In the following, we wish to discuss the rate at which the organic phosphorus compounds present in the muscles of the frog are renewed. We shall consider those cases of renewal[1]

[1] It is conceivable that molecules are renewed whithout the splitting off and reincorporation of phosphate group.

incorporated into the organic molecules. For example, creatinephosphoric acid is degraded under splitting off of phosphate and resynthesized under uptake of phosphate radicals. If labelled phosphate ions are present, they will have the same chance to be incorporated as have non-labelled ones. Let us assume 10^6 free phosphate ions present in the muscle cells to contain 10 $^{32}PO_4$ ions while from 10^6 P atoms isolated from hexosemonophosphate of the muscle tissue only 1 is ^{32}P, then we have to conclude that 10 per cent of the hexosemonophosphate molecules were renewed during the experiment under incorporation of free phosphate. The ratios of the specific activities of the inorganic P and the organic P are, thus, a measure of the extent of renewal of the organic P compound which took place during the experiment. When trying to arrive at quantitative data we encounter the following difficulties: (a) The free phosphate extracted from the muscle tissue is partly cellular and partly extracellular phosphate; it is, however, the specific activity of the cellular phosphate only which is to be considered when calculating the rate of renewal. (b) The specific activity of cellular phosphate changes during the experiment, the change being due, for example, to an increasing influx of labelled phosphate into the muscle cells. In this connection it should also be mentioned that the method permits to distinguish between renewed and non-renewed, between "old" and "new" molecules; but no information is supplied on the point whether the molecules are repeatedly renewed in the course of the experiment or not.

As to point (a), to account for the share of the extracellular phosphate in the total phosphate of the muscle tissue, we must know the specific activity of the plasma phosphate which we assume to be identical with the specific activity of the extracellular phosphate. We must also know the phosphate content of the plasma and that of the muscle tissue and, finally, the size of the extracellular space. The last mentioned magnitude can be determined in each case by administering simultaneously with the labelled phosphate labelled sodium[1], or it can be assumed that the extracellular space makes out 14 per cent of the weight of the muscles. Another procedure which we used repeatedly is the following. We remove one leg of the frog 1 hour after the start of the experiment and determine the specific activity of the free muscle phosphate P. After further 3 hours, we extract the phosphate of the other gastrocnemius and determine the specific activity of the free phosphate P. If, within 1 hour, a proportional partition of ^{32}P between plasma phosphate and the extracellular phosphate took place, then the increment of the specific activity of the tissue phosphate between 1 hour and 4 hours is solely due to an increase in the specific activity of the cellular inorganic P. By this method, we can determine the percentage of cellular P which was replaced in the muscle

[1] Comp. G. Hevesy and O. Rebbe, *Acta Physiol. Scand.* **2**, 171 (1940).

by plasma P between 1 hour and 4 hours after the start of the experiment.

The fact that the inorganic P of the tissue is partly of extracellular origin will lead to an overestimation of the activity of the cellular inorganic P and, thus, to an underestimation of the renewal figures of the organic P compounds. This source of error is mainly to be considered in experiments of short duration carried out at low temperature. On the other hand, even if the greatest precautions are observed, we risk a decomposition of some of the creatine phosphate present in the tissue prior to the separation of the inorganic P. Such a decomposition will lead to a decrease in the specific activity of the inorganic P, the inorganic P originating from creatine P being on the whole less active than the "free" phosphate P. We shall, thus, underestimate the specific activity of the inorganic P and, correspondingly, overestimate the rate of renewal of the organic P compounds. This error will also be larger in experiments of short duration carried out at low temperature. We wish to mention a further possible experimental error. If the free phosphate is not precipitated quantitatively, we risk to find some strongly active phosphate in the creatine phosphate fraction. A non-negligible amount of phosphate may remain in solution in cases in which the amount of P to be precipitated is very small.

The following objection can be put forward regarding the calculation of renewal rates from the ratio of the specific activities of the inorganic P and the P split off from organic compounds. The P secured as inorganic phosphate might even after the most careful handling of the tissue have been largely present not as free phosphate in the tissue cells but incorporated in very labile compounds which were decomposed in the course of the extraction process. It is possible that this is the case, it is even quite possible that a large part of the inorganic P extracted as such from the muscle cells was originally present incorporated in very labile compounds and was decomposed during the extraction process. General experience indicates, however, that very labile phosphorus compounds are renewed at a fast rate and we can, therefore, expect the P of such labile phosphorus compounds to obtain within a short time a similar specific activity as shown by the inorganic P present in the cells. Should that not be the case, then the comparison of the specific activity of the "inorganic" P with that of the P split off from the organic compound in question, would obviously lead to an overestimation of the rate of renewal.

The specific activity of different P fractions is seen in Tables 11—18.

Though all precautions were taken to prevent decomposition of creatinephosphoric acid it is difficult to state whether the variations in the values obtained for the rate of renewal of creatinephosphoric acid molecules in some of the experiments are genuine or are due to a more

or less successful prevention of the decomposition of the creatinephosphoric acid prior to the removal of the inorganic phosphate of the muscle tissue.

The results of an experiment, in which the frog was kept at 20° for

TABLE 11. — SPECIFIC ACTIVITY OF PHOSPHORUS FRACTIONS EXTRACTED FROM THE GASTROCNEMIUS OF A FROG, 4 HOURS AFTER INJECTING LABELLED SODIUM PHOSPHATE INTO THE LYMPH SACK. TEMP.: 2°

Fraction	P content in mgm	Activity in per cent of the standard preparation	Per cent of total activity administered per mgm P	Relative specific activity
I. Inorganic P	0.313	69.5	0.888	100
II. Inorganic + creatine P	0.935	100	0.428	48.2
III. Creatine P calculated as II—I	0.622	30.5	0.20	22.1
IV. Creatine P (isolated)	0.362	17.1	0.19	21.4
V. Pyrophosphate + hexose P	0.215	8.3	0.16	17.4
VI. Acid soluble residual P	0.113	3.2	0.11	12.7
VII. Non acid soluble P	0.500	1.6	0.013	1.4

We denote as pyrophosphate + hexose P the inorganic phosphorus obtained after the hydrolysis of a fraction for one hour at 100° in 1 *N*. H_2SO_4 after the removal of the inorganic and creatine P.

4 hours and then for 1 hour at 0°, is seen in Table 18. The muscles were immersed in liquid air and treated with cold 5 per cent trichloracetic acid. The extract was sucked through a glass filter into cooled FISKE's reagent. These operations took 2 minutes. In this experiment, we tested

TABLE 12. — SPECIFIC ACTIVITY OF PHOSPHORUS FRACTIONS EXTRACTED FROM THE GASTROCNEMIUS OF FROGS. TEMP.: 2°

Time of the experiment hours	Fraction	Specific activity
4	Inorganic P	100
4	Creatine P	14.8
4	Inorganic P	100
4	Creatine P	21.4
3	Inorganic P	100
3	Creatine P	8.8

to what extent the inorganic P is precipitated by FISKE's reagent. After precipitation of the "free" phosphate, 60 mgm sodium phosphate were dissolved in the filtrate, the phosphate was then precipitated and its activity tested. If the first precipitation was strictly quantitative, this second precipitate should be inactive. The counter registered 228 counts while, in the case of the first precipitate, 2500 counts were regis-

tered. When the 228 non-precipitated counts are considered, the specific activity of the creatine P fraction works out to be 14.1 instead of 15.6.

The same technique was used in the following experiments.

The lowest value found for the percentage renewal of creatinephosphoric acid molecules in the course of 4 hours at 0° is 9 and in the course of 17.5 hours 10 while, in most experiments, appreciably larger figures were found. The rate of renewal of the creatinephosphoric acid molecules

TABLE 13. — SPECIFIC ACTIVITY OF PHOSPHORUS FRACTIONS EXTRACTED FROM THE GASTROCNEMIUS OF A FROG 40 HOURS AFTER INJECTING LABELLED SODIUM PHOSPHATE. TEMP.: 2°

Fraction	Relative specific activity
I. Inorganic P	100
II. Inorganic + creatine P	62.7
III. Creatine P (calculated as II—I)	33.7
IV. Creatine P (determined)	34.4
V. Pyrophosphate + hexose P	22.8
VI. Acid soluble residual P	11.3
VII. Non acid soluble P	2.1

TABLE 14. — SPECIFIC ACTIVITY OF PHOSPHORUS FRACTIONS EXTRACTED FROM THE GASTROCNEMIUS OF A FROG 24 HOURS AFTER INJECTING LABELLED SODIUM PHOSPHATE. TEMP.: 20°

Fraction	Relative specific activity
Inorganic P	100
Creatine P	95
P hydrolysed in the course of 1 hour	91
Acid soluble residual P	36

TABLE 15. — SPECIFIC ACTIVITY OF PHOSPHORUS FRACTIONS EXTRACTED FROM THE GASTROCNEMIUS OF A FROG 400 HOURS AFTER INJECTING LABELLED SODIUM PHOSPHATE. TEMP.: 20°

Fraction	Relative specific activity
Inorganic P	100
Creatine P	99
P hydrolysed in the course of 1 hour	87
Non acid soluble P	16

TABLE 16. — SPECIFIC ACTIVITY OF THE PHOSPHORUS FRACTIONS OF THE MUSCLES OF A FROG KEPT AT 20° FOR 4 HOURS AND SUBSEQENTLY AT 0° FOR 1 HOUR

Fraction	P content in mgm	Counts per min.	Specific activity	Relative specific activity
		Left gastrocnemius		
Inorganic P	0.055	427	7770	100
Creatine P	0.240	290	1210	15.6
Hexose P	0.143	98	686	8.7
		Right gastrocnemius + sartorius		
Inorganic P	0.350	2500	7150	100
Creatine P	0.908	1010	1110	15.6
Residue after 17 hours hydrolysis	0.372	45	120	1.68

of the resting frog is, thus, quite appreciable even at 0° though not as high as stated in a preliminary note[1]. At 20° the lowest figure found after 4 hours is 16 per cent.

Rate of interaction of the plasma phosphate and the cellular phosphate of the muscle tissue

In the preceding section, we calculated the rate of renewal of the organic P compounds present in the muscle tissue by comparing the ^{32}P content of the tissue inorganic P with the ^{32}P content of the phos-

TABLE 17. — SPECIFIC ACTIVITY OF THE PHOSPHORUS FRACTIONS OF THE MUSCLES OF 2 FROGS KEPT AT 0° FOR 17.5 HOURS

Frog	Fraction	P content	Specific activity
I Gastrocnemius	Inorganic P	0.330	100
	Creatine P	0.474	29.8
	Product of 100 min hydrolysis	0.226	27.6
	Residue after 120 hours hydrolysis	0.070	2.0
I Sartorius	Inorganic P	0.799	100
	Product of 7 min hydrolysis	1.056	25.1
	Product of 17 hours hydrolysis	0.758	19.5
	Residue	0.246	6.8
II Gastrocnemius	Inorganic P	0.398	100
	Creatine P	0.452	11.6
II Sartorius	Inorganic P	0.475	100
	Creatine P	0.694	10.1

[1] G. HEVESY and O. REBBE, *Nature* **141**, 1097 (1938).

TABLE 18. — SPECIFIC ACTIVITY OF PHOSPHORUS FRACTIONS ISOLATED FROM DIFFERENT ORGANS OF A FROG, AFTER ADMINISTRATION OF LABELLED PHOSPHATE DURING 45 HOURS AT 20°

Fraction	Specific activity
Plasma P	100
Corpuscle P	3.6
Gastrocnemius inorganic P	4.9
Gastrocnemius creatine + pyrophosphate P	5.3
Liver P	10.1
Epiphysis P	0.35
Diaphysis P	0.20

TABLE 19. — SPECIFIC ACTIVITY OF PHOSPHORUS FRACTIONS ISOLATED FROM DIFFERENT ORGANS OF A FROG AFTER ADMINISTRATION OF LABELLED PHOSPHATE DURING 4 DAYS AT 22°

Fraction	Specific activity
Plasma P	100
Gastrocnemius inorganic P	8.3
Gastrocnemius creatine P	7.4
Sartorius total acid soluble P	8.5
Gastrocnemius phosphatide P	1.5

phorus extracted from the compound in question. In the following, we shall discuss the interaction of the plasma phosphate with the cellular phosphate. This is clearly a very different problem, the rate of interaction between the plasma phosphate and the cellular phosphate being determined by the permeability of the cell membrane.

The low rate at which phosphate ions migrate through the membrane of the cells of the gastrocnemius is seen in Tables 18 and 19. In the course of 4 days at 22° only somewhat less than 1/10 of the P atoms present in the labile P compounds got replaced by plasma P. The molecules of the labile P compounds were repeatedly renewed during this interval and many P atoms present in the muscle cells interchanged lively; however, the interchange between cellular and extracellular P took only place on a restricted scale.

The results of further experiments in which the activity of the plasma was compared with the activity of the muscle is seen in Table 20.

To keep the plasma activity at an approximately constant level throughout the experiment, 0.4 cc. solution was injected at the start of the experiment and further 0.08 cc. every hour. As seen in Table 20, within 1 hour and 4 hours the activity of the plasma changes only

slightly, the average being 108, taking the end value to be 100. The values obtained for the specific activity of the tissue P are seen in Table 20 and Fig. 3.

In some cases, very low values were obtained for the distribution ratio of labelled phosphate between plasma and muscle tissue. The fact

FIG. 3. Specific activity of tissue P.

that in these experiments frogs kept through the winter were used, the experiment being carried out in the spring, suggests the explanation that poor circulation may be responsible for the low values obtained.

TABLE 20. — ACTIVITY OF DIFFERENT FRACTIONS OF THE FROG 1 HOUR AND 4 HOURS, RESPECTIVELY, AFTER THE START OF THE EXPERIMENT

Temp.: 22°

Time	Fraction	Fresh weight in mgm	P content in mgm	Activity per mgm fresh weight	Specific activity of P
1 hour	Plasma	98.7	0.00344	116[1]	116
	Gastrocnemius	861.8	1.355	32.7	0.72
	Epiphysis	94.1	6.100	224	0.12
	Diaphysis	67.6	6.830	235	0.08
4 hours	Plasma	1267.5	0.044	100	100
	Gastrocnemius	812.4	1.300	93.5	2.03
	Epiphysis	38.8	3.61	755	0.28
	Diaphysis	60.1	6.410	578	0.19

[1] Taking the 4 hours value to be 100.

Increment in the Specific Activities between 1 Hour and 4 Hours

Fraction	Experimental value	Value corrected for the change in the specific activity of the plasma
Gastrocnemius	1.34	1.21
Epiphysis	0.16	0.148
Diaphysis	0.11	0.102

^{32}P content of the liver fractions

As in the case of mammalia, in the frog the liver phosphate interacts at a much faster rate with the plasma phosphate than does the muscle phosphate. The fast rate of renewal of the acid soluble P compounds

Table 21. — Specific Activity of the Phosphorus Fractions Extracted from the Organs of the Frog Kept at 15° and the Organs of the Rabbit 10 Hours after Administration of Labelled Phosphate

Fraction	Specific activity	
	Frog	Rabbit
Plasma	100	100
Gastrocnemius inorganic P	2.11	15.5
Gastrocnemius total acid soluble P .	1.49	11.0
Liver inorganic P	12.9	85
Liver pyrophosphate P	15.2	—
Liver hexosemonophosphate P	8.9	—
Liver residual acid soluble P	3.5	—
Liver phosphatide P	0.04	12.8

Table 22. — Distribution of ^{32}P between Plasma and Corpuscles of the Frog

Time of experiment hours	Temp.	Distribution ratio of ^{32}P between corpuscle and plasma of equal weight
10	15°	0.28
14	20°	1.1
45	20°	3.6

and the very slow renewal of the phosphatides of the liver of the frog are seen in Table 21. This table contains also corresponding data for the P fractions of the rabbit.

^{32}P content of the red corpuscles

As seen in Table 22, a very slow interaction was found to take place between the plasma P and the corpuscle P present in the nucleated corpuscles of the frog.

Summary

The rate of absorption of phosphate injected into the lymph sack of the frog was studied using radiophosphorus as an indicator. The maximum amount of labelled phosphate present in the circulation after subcutaneous injection at any moment was found to be 3 to 4 per cent of the amount administered, thus a similar value as found in the case of mammalia.

While at 2° after the lapse of 1 to 3 weeks 1 gm bone tissue contained about 15 times as many labelled P atoms as 1 gm muscle tissue, the corresponding ratio was found to be but 3 at 22°, showing that the temperature coefficient of the penetration of labelled phosphate into the muscle cells followed by incorporation of labelled P into the phosphorus compounds of the muscle tissue is much larger than the temperature coefficient of the formation of labelled bone apatite crystals.

The amount of labelled phosphate excreted by the kidneys and the amount of labelled phosphate taken up by the frog kept in physiological sodium chloride solution containing labelled phosphate were investigated.

The rate of renewal of various acid soluble P compounds extracted from the gastrocnemius of the frog was determined by comparing the specific activity of the inorganic P extracted from the muscle with the specific activity of the phosphorus split off from various organic compounds of the muscle tissue. Creatine-phosphoric acid molecules, adenosintriphosphoric acid molecules, and also hexose-monophosphate molecules were found to be renewed at an appreciable rate even at 0°. The rate of renewal was found to increase with decreasing chemical stability of the compound and with increasing temperature.

The rate of interaction of the plasma phosphate with the phosphate of the muscle cells was found to be very much lower than the rate of interaction of the free cellular phosphate with the phosphate of several organic phosphorus compounds.

The rate of penetration of labelled phosphate into the liver cells is much faster than the rate of penetration into the muscle cells. The rate of interchange of plasma phosphate and the phosphate of the corpuscles was found to be fairly low.

Comments on papers 36—38

In co operation with Parnas (1938) in *in vitro* experiments, the incorporation of the phosphate group into various acid-soluble phosphorus compounds was studied. It was found that a phosphate group may be transferred from one organic molecule to another without passing the inorganic stage. For example, when glucose-1 phosphoric acid (Cori ester) is transformed in the presence of muscle extract and labelled inorganic phosphate into glucose-6-phosphoric acid (Robison ester) the esters do not become labelled.

Simultaniously Meyerhof, *et al.* studied the rate of interchange between inorganic and pyrophosphate P in muscle-extract and found that after the lapse of 20 sec, a 47 per cent interchange took already place.

The first *in vivo* studies on the turnover of acid-soluble phosphorus compounds of the muscle tissue were carried out with frogs (paper 36). The turnover rate of the acid-soluble constituents was found to be influenced to a much higher degree by temperature- changes than by the formation of the labelled bone apatite crystals. When investigating the rate of renewal of the acid-soluble organic phosphorus compounds of the rabbit (paper 37), the inorganic phosphate content of the plasma was kept at an almost constant level throughout the experiment, which took 50 days. This made it possible to arrive, by comparison of the specific activities of the cellular inorganic P and the organic P at the end of the experiment, at a renewal figure of the latter. In experiments of 50 days duration, the results can be expected not to be influenced much by the participation of slowly-formed intermediary products, or the specific activity of extracellular phosphate much to differ from that of the intracellular one, in contrast with experiments taking a few hours only.

Recently in ^{18}O of $H_2{}^{18}O$ a very suitable indicator in the study of the turnover of phosphorus compounds of the muscle cells was found by Fleckenstein *et al.*

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39. TURNOVER RATE OF THE FATTY ACIDS OF THE LIVER

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The rate of renewal of the fatty acids of the liver and other organs has been repeatedly determined by making use of deuterium, carbon 13 and carbon 14 as an indicator. In early experiments Schoenheimer and Rittenberg[1] administered heavy water to mice and kept the deuterium content of body fluids at a constant level throughout the experiment. The saturated fatty acids of the mouse reached half of their maximal deuterium content in the time of 5 to 9 days. The deuterium content of the saturated acids was higher than that of the unsaturated.

Bernhard and Schoenheimer[2] isolated the fatty acids of the intestinal wall, the kidneys and the liver of the mouse and found the saturated fatty acids to have an appreciably higher deuterium[3] content than had that of the unsaturated ones. They estimate the half-life time of the average saturated fatty acid molecule in the liver of the mouse to be about 1 day, while the half-life time of the total fatty acids in the rat liver was found by Stetten and Boxer[4] to be 1.9 days.

The finding of Rittenberg and Bloch[5] that the feeding to mice of acetate containing ^{13}C in the carboxyl group leads to the formation of fatty acids containing ^{13}C, opened the way to the application of ^{13}C and ^{14}C in the study of the rate of formation of fatty acids. They found a more rapid incorporation of ^{13}C in to the saturated than into the fatty acids. The ^{13}C concentration of the carboxyl carbon atoms of the saturated fatty acids was approximately twice as high as the average of all the carbon in the saturated fatty acids. The most plausible distribution which will explain these data is one in which the labelled carbon is

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[4] D. Stetten Jr., and G. E. Boxer, *J. Biol. Chem.* **155**, 231 (1944).
[5] D. Rittenberg and K. Bloch, *J. Biol. Chem.* **154**, 311 (1944). K. Bloch and D. Rittenberg, *J. Biol. Chem.* **159**, 45 (1945). D. Rittenberg and K. Bloch, *J. Biol. Chem.* **160**, 417 (1945).

present at every other carbon atom; i.e. at the odd number carbon atoms of the fatty acids. Later work[1] showed that at least 25 percent of the carbon atoms of the fatty acids are derived from acetate. Evidence was also obtained that acetic acid can furnish every carbon atom of the molecule.

Recently the rate of turnover of fatty acids has been re-investigated with the aid of acetic acid labelled by ^{14}C in the carboxyl group by Pihl *et al.*[1] The percentage renewal of the fatty acid molecules is determined by

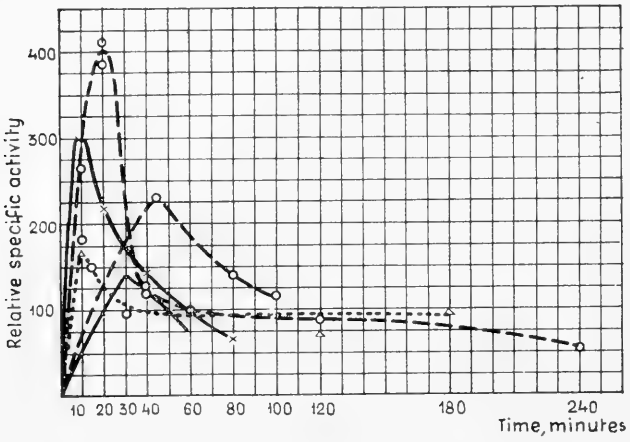

Fig. 1. Change of the specific activity of the liver fatty acids with time.

comparing the ^{14}C content of the fatty acid carbon at the end of the experiment with the average value of the ^{14}C content of the precursor carbon which prevailed during the experiment. To arrive at the last mentioned data, phenyl-DL-aminobutyric acid was fed simultaneously with labelled acetate to adult rats kept on fat free diet, and consecutive samples of the excreted acetyl derivatives were analysed.[3] Though the labelled acetate content of the diet was kept constant, the isotope concentration of the acetyl group was found to increase in the course of the 30 days period with about 30% of the initial value. This increase was shown to be due to the catabolism of the labelled higher fatty acids formed during the experiment. The metabolic products of the labelled

[1] K. Pontecorvo, D. Rittenberg and K. Bloch, *J. Biol. Chem.* **179,** 893 (1949). A. Pihl, K. Bloch and H. S. Anker, *J. Biol. Chem.* **183,** 441 (1950).

[2] K. Pontecorvo, D. Rittenberg and K. Bloch, *J. Biol. Chem.* **179,** 893 (1949).

[3] K. Bloch and D. Rittenberg, *J. Biol. Chem.* **159,** 45 (1945).

fatty acids contribute in these long-time experiments significant quantities of labelled acetyl groups to the acetic acid pool which supplies ^{14}C to the newly formed fatty acid molecules.

The saturated fatty acids of liver were found to reach half of their maximal isotope concentration in less than 1 day, the unsaturated acids in about 2 days. Much longer time is necessary to reach a corresponding ^{14}C concentration in the fatty acids of the carcass, 16—17 days for saturated and 19—20 days for the unsaturated acids. This difference was also shown in recent work of POPJAK and BEECKMANS[1].

In an investigation on the effect of changes in the metabolic rate on the incorporation of ^{14}C into tissue fractions, we determined the specific activity of the liver fatty acids of the mouse following injection of carboxyl labeled acetate in experiments of 10 to 180 min duration. The results obtained, which are discussed in this note, suggest, the existence of a fatty acid fraction in the mouse liver of much shorter half-life than about 1 day, which was found in the various experiments mentioned above.

EXPERIMENTAL

In each experiment 45 to 72 mice were injected intraperitoneally with 0.2 ml of 0.8% sodium chloride solution containing 2—4 microcurie (0.2—0.4 mgm) of sodium acetate labelled in the carboxyl group. The animals were divided in 5—6 equal groups and killed after 5 to 240 min.

The pooled organs were frozen in solid CO_2. The ground tissue was then extracted for 3 hr with a boiling mixture of ether-alcohol 1: 3. The filtrate obtained was evaporated *in vacuo* and the residue extracted with petroleum-ether. The residue obtained after evaporation of the petroleum-ether was saponified for 8 hr with 10 ml of 40% KOH solution and 20 ml of alcohol on a boiling waterbath.

The solution was extracted three times with petroleum-ether in order to remove the insaponifiable matter. The aqueous solution was then neutralised with 40% H_2SO_4 solution and extracted three times with petroleum-ether.

The petroleum-ether solution on evaporation gave the fatty acids. The determination of the radioactivity was carried out with a Geiger counter, 8 mgm of each sample on an aluminium disk of 5 mm diameter.

[1] G. POPJAK and M. L. BEECKMANS, *Biochem. J.* **69**, 547 (1950).

DISCUSSION

The relative specific activity of the total fatty acids extracted from the liver of mice killed at different times after intraperitoneal injection of acetate labelled in the carboxyl group is plotted in Fig. 1. In view of the fact that the five experiments, the results of which are recorded, were carried out with different strains of mice, we plotted the results of each experiment by taking the value obtained after 60 min experiment to be 100. Each point indicates a value obtained by extracting the fatty acids of to 12 pooled livers. The total number of mice involved in these experiments amounted to 250. 1 mgm of fatty acid of the liver of a 20 gm mouse contains 8.2×10^{-4} part of the ^{14}C administered. This figure indicate thes value corresponding to the highest peak of the curves.

It takes several minutes until the injected labelled acetate or its decomposition products penetrate into the liver and are incorporated into fatty acid molecules. Correspondingly, the specific activity of the fatty acids increases for the first minutes. This time was determined for the rat to be 15 min.[1] The increase in the specific activity of the fatty acid precursors with time is soon followed by a decrease. While the endogenous inactive acetate is constantly being produced, about 1·2 mgm are formed daily per 100 mgm of rat tissue[2], the injected labelled acetate is not replaced. Correspondingly, after a while fatty acid molecules will be synthesized in the liver from almost inactive precursors. The time will also arrive when the labelled fatty acid molecules present will be renewed a second time from less active precursors than the first time. Active fatty acid molecules will thus be replaced by less active ones, and the specific activity of the average fatty acid molecule shall now decrease with time. The rate of decrease will be determined by the half-life time of the liver fatty acid molecules. Let us assume that after the lapse of 30 min. no further active fatty acid molecules are formed in the liver, the precursors being no longer active, and correspondingly the specific activity of the fatty acid of the liver will decrease with time according to the formula

$$S_t = S_o e^{-\frac{\ln 2\, t}{T}}$$

where S_0 denotes the specific activity after 30 min., S that at any later time t, while T = half-life time of the fatty acid molecules in the liver.

Assuming $T = 1$ day as found by different workers in feeding experiments, than in the interval between 30 and 60 min. the decline in the

[1] R. G. Gould, F. M. Sinex, I. N. Rosenberg, A. K. Solomon and A. B. Hastings, *J. Biol. Chem.* **177,** 295 (1949).

[2] K. Bloch and D. Rittenberg, *J. Biol. Chem.*, **159,** 45 (1945); A. Pihl K. Bloch and H. S. Anker, *J. Biol. Chem.* **183,** 441 (1950).

specific activity should be about 3% only. We assumed in the above calculation that all formation of labelled fatty acid molecules ceases after the lapse of 30 min. As this assumption does not hold strictly, the decline in the specific activity of fatty acids in the interval between 30 and 60 min. is even less than 3%.

The data of Fig. 1 indicate a very much larger decrease in the specific activity of the fatty acids than 3% in the interval between 30 and 60

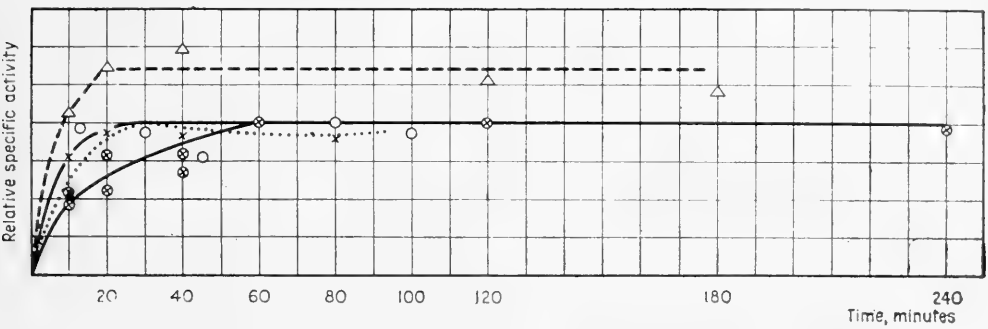

FIG. 2. Change of the specific activity of the brain fatty acids with time.

min after administration of the labelled acetate. The decrease amounts to about 50%. These results suggest the presence of a rapidly renewable fatty acid fraction in the liver of the mouse.

In experiments in which the labelled acetate is fed for days and the specific activity of the fatty acids in the liver daily determined, the presence of a minor fraction of rapid renewal rate cannot be expected to be observed.

We investigated also the change of the specific activity of the brain fatty acids and muscle fatty acids with time. The specific activity of the brain fatty acids was found, as seen in Fig. 2, to increase rapidly with time in the course of the first minutes and then to remain almost constant. We lack thus any indication of the presence of rapidly renewed fatty acid faction in the brain. The specific activity values of the muscle fatty acids extracted from different groups of mice fluctuate considerably. An indication of a decrease of the specific activity figures within a 240 min. observation period is, however, also in this case absent.

Summary

Following the injection of in its carboxyl group labelled acetate to mice, the specific activity of the fatty acids extractes from the liver after the lapse of 30 minutes is found to be appreciably lower than measured a few minutes after injection. It follows from this finding that the fatty acids of the liver contains a rapidly renewable fraction.

Originally communicated in *Arch. int. pharmacodyn.* **86,** 33 (1951)

EFFECTS OF DINITRO-CYCLO-PENTYLPHENOL ON THE INCORPORATION OF LABELLED ACETATE CARBON (^{14}C) INTO TISSUE FRACTIONS

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As found in studies in which labelled acetate (^{14}C) was used as an indicator, acetate is rapidly metabolized in the animal (1) body. While the bulk of the administered acetate carbon is soon exhaled, some is incorporated into tissue fractions.

Not only does the amount of administered labelled acetate diminish rapidly with time, but the specific activity of the body acetate diminishes as well. This is due to the constant new formation of endogenous non active acetate, which is, at least in the early phases of the experiment, practically inactive.

As an effect of the diminution of the specific activity of the body acetate, the sensitivity of the radioactive indicator strongly increases with time. One count of acetate ^{14}C which indicates the presence of 1 microgram of acetate or degradation product of acetate, at an early phase of the experiment may indicate the presence of 10 micrograms at a later phase.

A change in the metabolic rate of acetate or its degradation products reflects itself in a change in their specific activity. Correspondingly a change in the metabolic rate will lead to a change in the ^{14}C content of tissue fractions and by following these changes we may conclude if and to what extent metabolic changes took place in the organ considered. If the CO_2 production is due to metabolic interference slowed down from 90 p.c. to 89 p. c., the incorporation of ^{14}C into tissue fractions may increase from 10 to 11 % thus with as much as 1/10.

Gould and assoc. (2) in experiments with adult rats, demonstrated the rapid disappearance of the injected labelled acetate carbon through exhalation as CO_2. The cumulative exhalation amounted in 4 hours to 87 % of the amount injected. After the lapse of 30 min about 1/3 was still present in organic form in the rat, 1/3 in inorganic form, while 1/3 is excreted as CO_2. The administration of labelled acetate does not interfere with the normal metabolic processes. Acetate is constantly produced and metabolized in the animal organism, the daily production

of acetate in a 100 gm rat amounting according to BLOCH and RITTENBERG and PIHL *et al.* (1, 3) to 15-20 mM.

The effects of X-rays on animal tissue was found, in experiments in which labelled acetate was administered to mice, to influence markedly acetate metabolism (4). This observation induced the investigation of the effects of metabolic inhibitors and accelerators on acetate metabolism.

Previous work with mice carried out by RUYSSEN, BEECKMANS and one of us (5) showed an increased incorporation of ^{14}C in the urethane injected animals.

In the present investigation the effect of a metabolic accelerator, that of dinitro-cyclo-pentylphenol (D.P.P.) was studied on ^{14}C incorporation into the total tissue and the total fat of various organs of the mouse. We choose dinitro-cyclo-pentylphenol as HEYMANS and CASIER (6) found this compound to stimulate cellular metabolism to a larger extent than any other dinitro-compound studied.

Dinitro-cyclo-pentylphenol being a metabolic accelerator its effect on cellular metabolism can be expected to reflect itself in the rate of ^{14}C incorporation into tissue fractions.

As shown by different authors, oxygen consumption is increased after administration of 2.4-dinitrophenol and similar compounds.

TERADA and TAINER (7) injected subcutaneously 20 mgm of 2—4 dinitrophenol per kgm to 33 gm. rats and found an increase of about 25 % in the oxygen consumption 20 min after injection. After the lapse of 2 hours, normal oxygen consumption was again observed. In the case of adult rats the same effect could be obtained by injection of 15 mgm per kgm body weight.

A slight stimulation of oxygen consumption was observed by LOOMIS and LIPMANN (8) in their experiments in which the effect of small amounts of dinitro-phenol on kidney homogenate metabolism was studied and a marked lowering of phosphorylation observed. The inhibition of coupling between oxidative processes and the formation of high energy bonds was found by these authors to be a conspicuous effect of dinitro-phenol.

EXPERIMENTS

Five groups of five adult mice each, were injected with 0.3 cc. of a solution containing 0.16% of sodium dinitro-cyclo-pentylphenol (20 mgm for 1 kgm of animal).

Five groups of controls were injected at the same time with 0.3 cc. of a physiological sodium-chloride solution.

Ten minuten afterwards all groups were injected intraperitoneally with 3 μ of sodium acetate labelled in the carboxyl group.

The animals were killed at definite times.

The same organs in the same group were combined and frozen in solid CO_2.

A small amount of them were immediately dried at 70° and measured as total tissue.

The ground organs were then extracted with a boiling mixture of ether-alcohol 1/3 for 3 hr. The filtrate obtained was evaporated and the residue extracted with petroleum-ether.

The total fats obtained by evaporation of the petroleum-ether were purified following the procedure of FOLCH and VAN SLYKE (9).

The determination of the radioactivity was carried out with a Geiger-Müller counter. The activity of the samples was determined without converting these into barium carbonate.

RESULTS AND DISCUSSION

a) Liver

The change in the relative specific activity of the total fat extracted from the liver of control mice and mice injected with dinitro-cyclo-pentylphenol with time is seen in Fig. 1(*a*). Each value indicates the ^{14}C content of a fat sample obtained from 5 pooled livers. After administration of labelled acetate, the incorporation of ^{14}C into fatty components first increases, soon however due to the very rapid turnover rate of a liver fatty fraction observed, the labelled fatty molecules will be replaced by newly formed ones. As these molecules are formed from a much less active medium — the specific activity of the liver acetate rapidly decreases with time — they are less active than the degraded or emigrated active fatty molecules. Due to these facts, the curve representing the change in the specific activity of the liver fat with time soon shows a decreasing trend. This is found to be the case already after the lapse of 7—15 min after the injection of labelled acetate.

If the rate of fat formation is influenced by the effect of the administered dinitro-cyclo-pentylphenol and accelerated in the first phase of the experiments, more ^{14}C can be expected to be incorporated into the fat of the liver of such animals than in those of controls. The left part of the curve will thus show a higher peak in the case of the mice treated with the dinitro-compound than in that of the controls. The right part on the curve on the other hand can be expected to show a steeper descent for the animals injected with the dinitro-compound. Such a behaviour is actually shown by the curves seen in Fig. 1 and also in those of Fig. 2 in which the change of the specific activity of the exhaled CO_2 is plotted against time, after injection of labelled acetate and succinate. Succinate

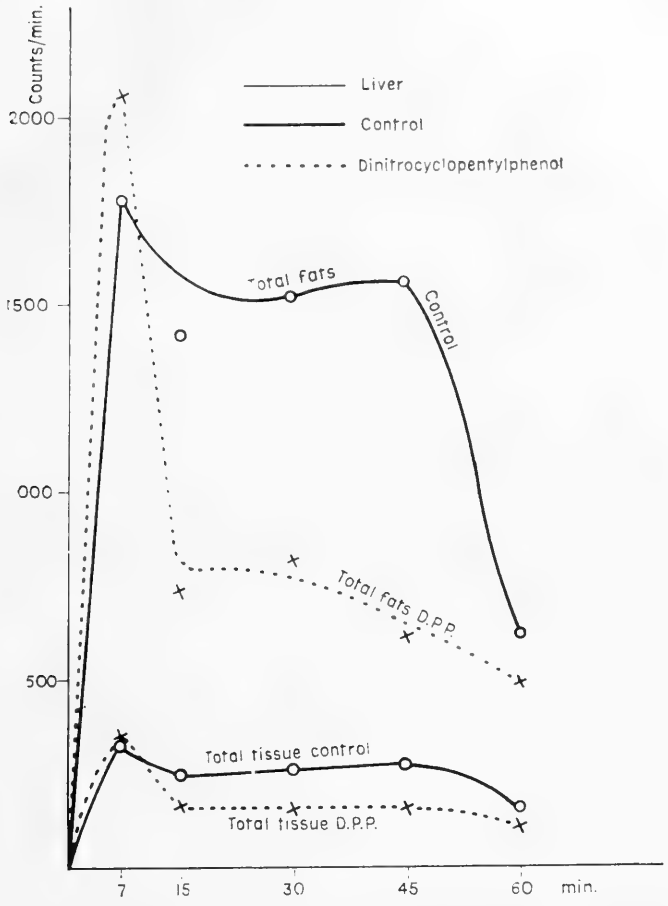

FIG. 1. Effect of dinitro-cyclo-pentylphenol on the incorporation of ^{14}C into total fats (*a*) and total tissue (*b*) of the liver of the mouse, ^{14}C injected as $CH_3{}^{14}COONa$.

is metabolized at a slower rate than acetate and correspondingly in the left part of the curve the acetate values are higher, in the right part of the curve the succinate values are higher (2).

A similar trend as indicated by the curves plotted in Fig. 1*a* is shown by the curves seen in Fig. 1*b* in which the specific activity of the dry liver tissue is plotted as function of time, both for controls and D.P.P. injected animals. The ^{14}C-content of the liver tissue except in a very early phase is mainly due to its fatty components but some is still present in acetic acid and other acid soluble tissue components, and to a very restricted extent in glycogen and in proteins. That the absolute ^{14}C content of the total tissue is much smaller than that of the fatty components, is due to the dilution of highly active fatty acids by large amounts of slightly active tissue components.

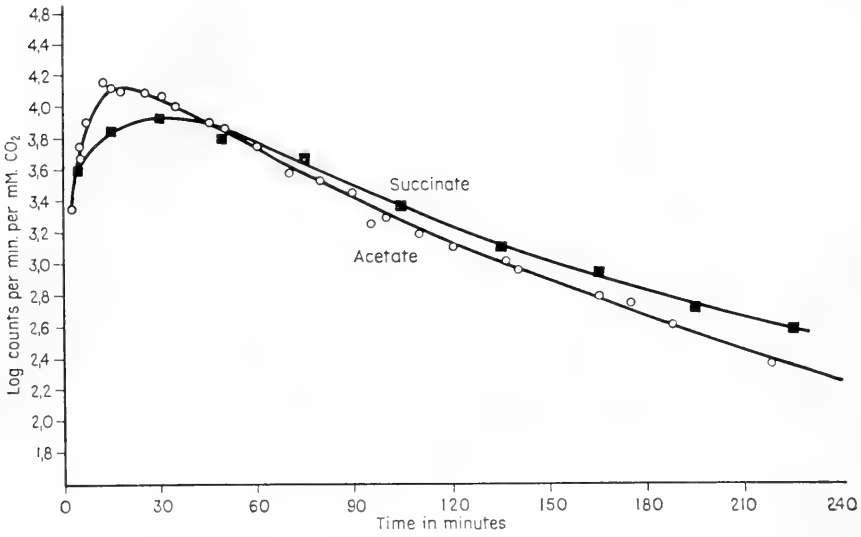

FIG. 2. The logarithm of the specific activity of CO_2 excreted is plotted against time, following the intraperitoneal injection of isotopic sodium acetate and succinate (GOULD *et al.* (2)).

b) Skeletal Muscles

A very different result as stated above is obtained in the study of the incorporation of ^{14}C into the total dry tissue of skeletal muscles, a large share of which is due to the fats present. As seen in Fig. 3, the administration of the dinitro-compound decreases the rate of incorporation of ^{14}C into the muscle tissue both in the initial and in the later phases of the experiment. Our very restricted knowledge as to fatty acid formation in the intrusion into the muscles and to that of other labelled compounds makes the interpretation of the above results difficult. If

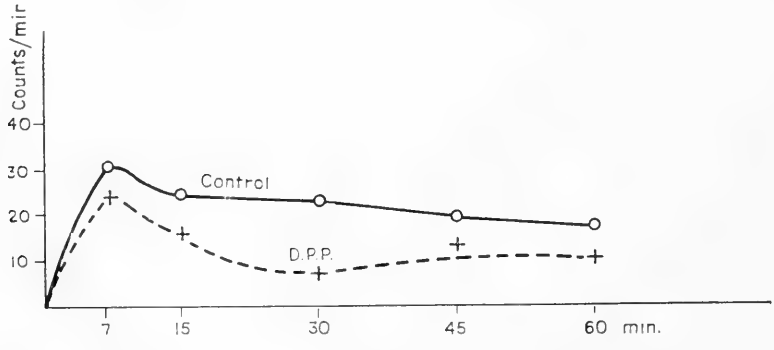

FIG. 3. Effect of dinitro-cyclo-pentylphenol on the incorporation of ^{14}C into the total muscle tissue of the mouse, ^{14}C injected as $CH_3{}^{14}COONa$.

under the action of the dinitro-compound the formation in or intrusion of fatty acids or their precursors into the muscle should be slowed down, we would obtain a lower ^{14}C content in the D.P.P. injected mice. A lower ^{14}C incorporation can, however, be the result not only of a depressed

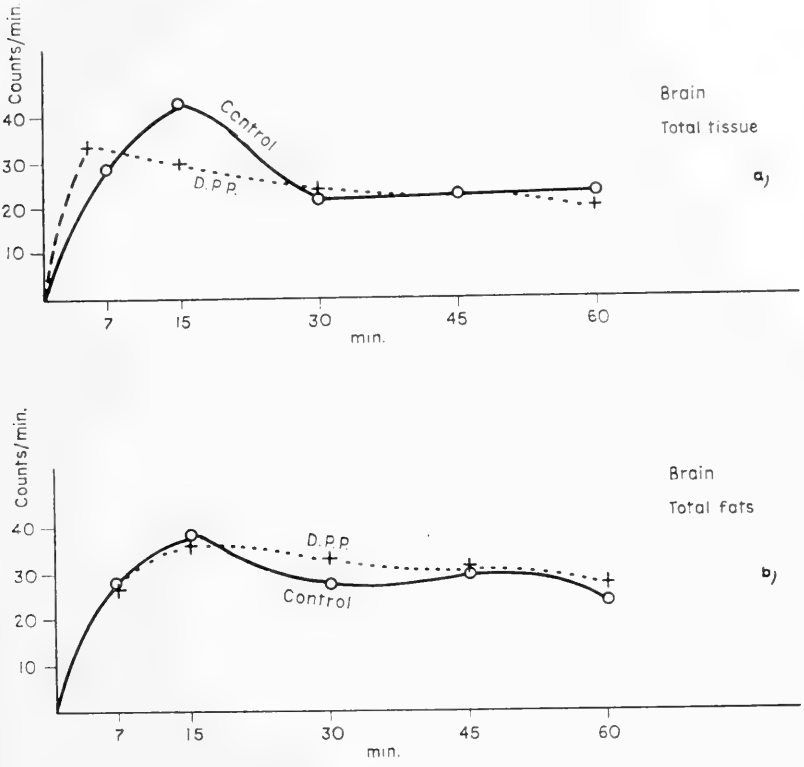

FIG. 4. Effect of dinitro-cyclo-pentylphenol on the incorporation of ^{14}C into total fats (*b*) and total tissue (*a*) of the brain of the mouse, ^{14}C injected as $CH_3{}^{14}COONa$.

formation rate of fats and other labelled components, but also of an enhanced dilution of the labelled acetate or its transportation products by endogenous inactive compounds.

Assuming the half-life time of the average fatty acid molecule in the mouse to be18 days, the metabolism of 1 gm fatty acids present in the muscles of a 20 gm mouse will lead to the formation of about 1 millimol endogenous (at the start inactive, later slightly active) acetic acid. In this calculation only the carcass fatty acids are considered (4). If under the effect of the D.P.P. the carcass fatty acids should be catabolized at an enhanced rate, for example, twice as rapidly as in the controls (inactivation of pyruvate is leading to the accumulation of acetate as well), the dilution of the active muscle acetate and its transformation products

would be twice as large in the D.P.P. treated animal. If the rate of formation of fatty acids would not be influenced in the muscles of such a mouse, the incorporation of ^{14}C into fatty acids would be half only in the D.P.P. injected mice of that of controls. Under physiological

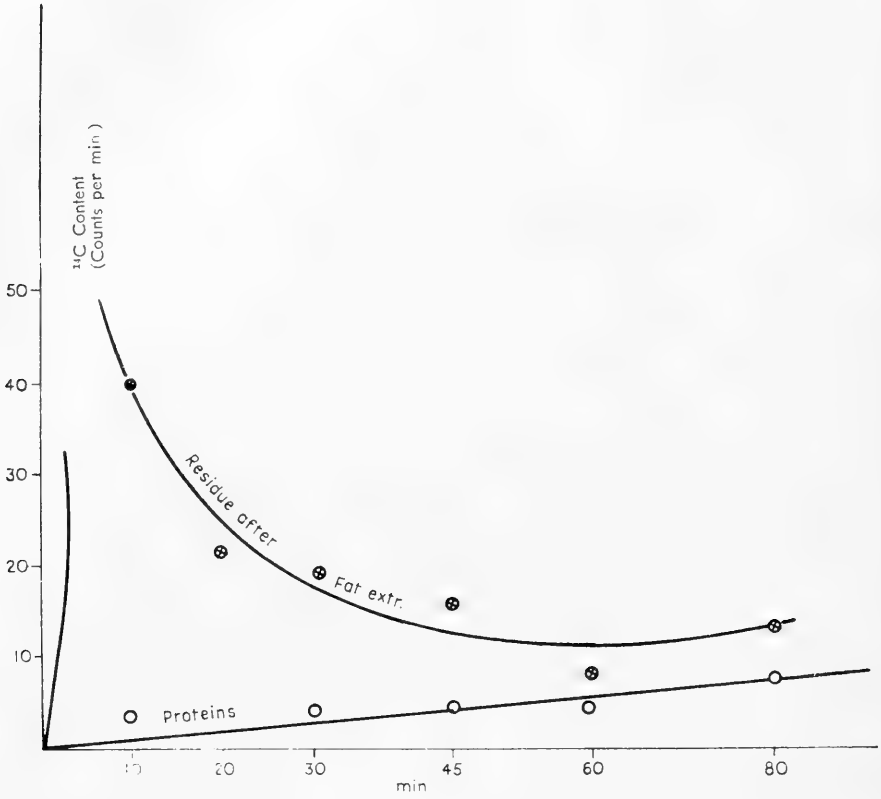

FIG. 5. Change of C^{14} content of brain fractions with time.

conditions the rate of formation of fatty acids will correspond to their rate of degradation. This is no longer the case in the D.P.P. treated animal which may rapidly loose some of its fat content.

It is quite possible that fats or their precursors reach the muscles from the liver. Most of the time the ^{14}C content of the fats in the liver of D.P.P. treated mice is lower than the ^{14}C content of the fats of controls. This may also explain the low ^{14}C values in the muscle fats of the dinitro-cyclo-pentylphenol treated mice. Further information on this point could be obtained by studying the intrusion into the muscles of labelled fatty acids, labelled aceto-acetonate or other labelled precursors introduced into the circulation.

c) Brain

The results plotted in Fig. 4(*a*) and 4(*b*) indicate a slight effect only of injection of D. P. P. on the metabolic steps of the brain in which acetate carbon participates. The total labelled acetic acid content of the rat

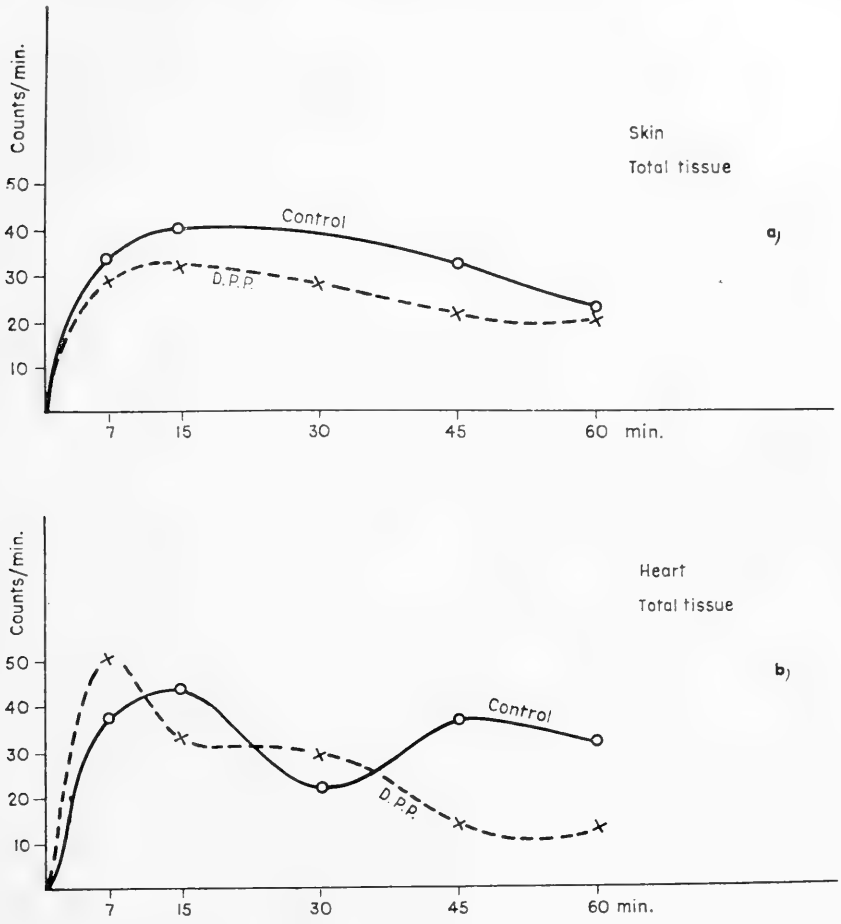

FIG. 6. Effect of dinitro-cyclo-pentylphenol on the incorporation of ^{14}C into total tissue of skin (*a*) and heart (*b*) of the mouse, ^{14}C injected as $CH_3{}^{14}COONa$.

was found by GOULD *et al.* (2) to decline during the same time-interval to 1/3 of its initial value as well. We can expect a very rapid metabolic rate in the brain in view of the high oxygen consumption of that organ. The striking decline in the activity of the fat free brain with time is seen in Fig. 5. After 30 min only, the activity of the acid, soluble and protein fractions is less than half of the value observed after 10 min. The slight increase in the ^{14}C content demonstrated by the last

part of the upper curve is presumably due to an increase in the activity of the protein fraction, which as shown by the lower curve is increasing with time.

d) Skin and Heart Muscle

For the skin and heart muscle we determined the effect of D.P.P. injection on ^{14}C incorporation into the total tissue only. The curves plotted in Fig. 6 (*a*) representing the results obtained for the skin, show a similar trend to the corresponding curves obtained for the skeletal muscle.

The first part of the curves which show ^{14}C incorporation into the heart tissue 6(*b*), indicates an accelerated rate of ^{14}C incorporation into the total organ under the effect of D.P.P. administration. The further trend of the figures is quite complicated, which may be due at least partly to the fact that we deal with the total tissue which contains fat and acid soluble fractions of appreciable ^{14}C content and less active protein and glycogen fractions.

The trend of the curve denoting the change in the specific activity of the heart muscle tissue under the effect of D.P.P. markedly differs from that of the curves obtained for the skeletal muscle. This difference suggests a specific effect of D.P.P. on the heart.

Summary

The rate of incorporation of ^{14}C after injection of carboxyl labelled acetate in the liver, brain, skin, skeletal and heart muscle of control mice is compared with the values obtained with organs of mice to which dinitro-cyclo-pentylphenol (D.P.P.) was administered.

Administration of D.P.P. increases the rate of incorporation of ^{14}C into liver fat and total liver tissue in the first 10 minutes of the experiment and decreases in the later part. Such a behaviour is expected if D.P.P. increases the rate of acetate metabolism in the liver.

Administration of D.P.P. has a slight effect only on ^{14}C incorporation into brain fat or total brain tissue.

The D.P.P. injected mice take up less ^{14}C in the skeletal muscle tissue and skin tissue than do the controls.

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Originally communicated in *Exp. Cell Res.* **3**, 191 (1952)

41. DETERMINATION OF THE RATE OF RENEWAL FROM THE RATE OF DISAPPEARANCE OF LABELLED MOLECULES

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University of Stockholm

The rate of renewal of a type of molecules is usually calculated from the rate of incorporation of the labelled atoms of the pertinent precursor in the molecules considered. If we wish, for example, to known the percentage of desoxyribonucleic acid molecules of the rat spleen, which are formed in the course of two hours, we administer labelled sodium phosphate to a rat and two hours later we compare the specific activities of the desoxyribonucleic acid P, and inorganic P extracted from the spleen. If the ratio of these specific activities is found to be 0.02, the specific activity of the inorganic P remained constant during the experiment, and this P can be considered to be the pertinent precursor of desoxyribonucleic acid P, we can conclude that in the tumour 2 per cent of the desoxyribonucleic acid molecules are present which were formed during the experiment or, more correctly, that at least 2 per cent of these molecules were formed in the course of two hours. If it takes sometime that the labelled precursor reaches the site of desoxyribonucleic acid syntesis the first phase of the synthesis of this compound will not be indicated by the tracer and we shall correspondingly underestimate its rate of synthesis formation.

If we protract the experiment, the specific activity of the inorganic P of the spleen decreases more and more and this decrease is followed by a decrease in the specific activity of desoxyribonucleic acid P. We can also calculate the rate of renewal by comparing the specific activities of desoxyribonucleic acid P and inorganic P in this declining activity phase of the experiment.

The rate of loss of ^{32}P by desoxyribonucleic acid molecules in the late phase of the experiment is independent of the precursor problem, but parallel with a loss ^{32}P by strongly active "old" desoxyribonucleic acid molecules the formation of less active "new" molecules takes place for example in the spleen and the rate of incorporation of ^{32}P in these molecules is partly determined by the specific activity of the pertinent precursor. Thus by replacing the calculation of the renewal rate from

increasing values of the specific activities of desoxyribonucleic acid with time by a calculation based on decreasing specific activity values with time, we cannot fully eliminate the difficulty arising from the lack of knowledge of the pertinent precursor of desoxyribonucleic acid P. We meet, however, very different conditions when faced with the task to calculate the rate of renewal of a carbon labelled compound from specific activity or similar data.

^{14}C of rapidly metabolized carbon compounds such as acetate, glucose and so on, is within a comparatively short time exhaled to a very large extent as carbon dioxide. Correspondingly the rate of loss of ^{14}C by fatty acid molecules in a later phase of the experiment in which, for example, labelled acetate was administered to the rat, is no longer a resultant of the disappearance of "old" strongly labelled molecules and the formation of "new" less strongly labelled ones but, at least in the first approximation, the result of the decay of labelled molecules only. By following the rate of decrease of the ^{14}C content of fatty acids extracted from the organs of the rat we can thus calculate the renewal rate of fatty acid molecules in an analogous way to that, in which we calculate the period of decay of a radioactive body from its "decay curve." In the present note the calculation of the renewal rate of the fatty acids of the liver from the rate of decrease of the ^{14}C content of the fatty acids in the rat injected with acetate labelled in the carboxyl group is described.

EXPERIMENTAL

44 rats weighing 190 to 244 gm were injected intraperitoneally each with 0.2 ml of phys. sodium chloride solution containing acetate labelled in the carboxyl group of 10—26 μ C activity. The animals were killed by decapitation and the total fat, fatty acids, neutral and phosphatide fatty acids, and also cholesterol of the liver, secured as described previously. (2, 8) The activity of the samples was compared without conversion into barium carbonate.

RESULTS

In an early investigation, using deuterium as an indicator, Stetten and Boxer (12) found the half life-time of fatty acids of the rat liver to amount to 1.9 days. Rittenberg and Bloch, (11) feeding ^{13}C labelled acetate, observed a more rapid incorporation of ^{13}C into saturated than into unsaturated fatty acids of the mouse liver. In a more recent work, Pihl *et al.* (8, 9) compared the ^{14}C content of the fatty acid carbon (at the end of the experiment taking many days) with the average value

of the ^{14}C content of the precursor carbon prevailing during the experiment. The saturated fatty acids of the liver were found to reach half of their maximum isotope concentration in less than one day, the unsaturated acids in about two days.

When investigating the effect of muscular exercise on the incorporation of ^{14}C into liver fats, the present writer (5) found after the lapse of $4\frac{1}{2}$ hr the ^{14}C content to be only one third of that detected 20 min after injecting labelled acetate into the mouse; however, according to the above mentioned data, in the course of two hr. the loss of ^{14}C by liver fatty acids should be less than 10 per cent. Obviously, a fatty fraction is present in the liver which has a much shorter half-life time than one day. RUYSSEN, BEECKMANS and the author (6) investigated the renewal rate of fatty acids in the liver of the mouse shortly after administration of labelled acetate. They obtained the result that the ^{14}C content of the liver fatty acids increase rapidly during about the first 30 minutes, this increase being followed by a rapid decrease. Furthermore, BEECKMANS and ELLIOTT (2) could demonstrate that in both the saturated and unsaturated fatty acid fractions this early increase is followed by a rapid decline in the ^{14}C content. The liver must thus contain one or more rapidly renewed fatty acid fractions. To discard the effect of this rapidly metabolizing fatty acid fraction we studied the decrease in the ^{14}C content of liver fatty acids one or $1\frac{1}{4}$ days after administration of labelled acetate only and followed it till the lapse of 4 or $4\frac{1}{4}$ days. As we in our determination of the renewal rate disregard those labelled fatty acid molecules which are formed during the experiment we have obviously quite apart from the above considerations to wait for about 1 day after injecting labelled acetate before securing the samples.

GOULD *et al.* (4) found that after the lapse of three hr. only 85 per cent of the acetate ^{14}C injected into the rat is already exhaled as $^{14}CO_2$. In the later phase of the experiment the rapid loss of the exogenous acetate ^{14}C may to some extent be compensated by formation of labelled endogenous acetate. PIHL *et al.* (9) kept the activity of body acetate of the rat at a constant level by feeding labelled acetate; in these experiments an increase in the body acetate activity could be observed after the lapse of ten days which was due to the formation of endogenous acetate of appreciable C^{14} content. Under our experimental conditions (injection of the labelled acetate at the start of the experiment) the activity of endogenous acetate formed during the experiment was, however, negligible.

In Fig. 1 the decrease in the specific activity of fatty acid carbon with time $1\frac{1}{4}$ to $4\frac{1}{4}$ days after injection of labelled acetate is plotted; also data obtained by PIHL *et al.* (9) for the increase in the percentage of labelled fatty acids with time are seen. The figure contains thus both "rise curves" and "decay curves". From the data of PIHL *et al.*

follow that the half life-time of saturated fatty acids is less than one day and that of unsaturated fatty acids about two days; our data indicate practically the same result, 0.8 and 2.2 days, respectively. A closer coincidence is hardly to be expected in view of the fact that even when comparing fatty acid turnover in the liver of rats of the same race, age and weight, very appreciable fluctuations appear. Ame-

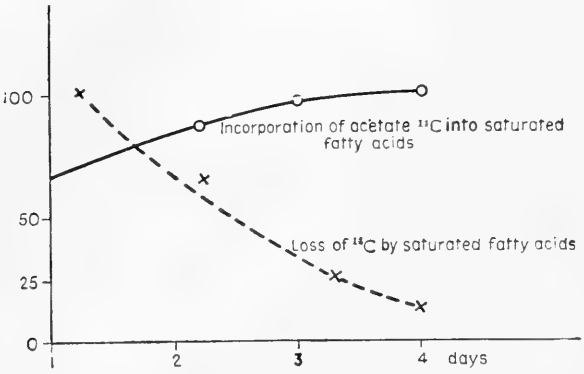

Fig. 1. Rate of incorporation of acetate C^{14} (Pihl *et al.*) and rate of loss of ^{14}C by saturated fatty acids extracted from rat liver. ("Rise curve" and "Decay curve".)

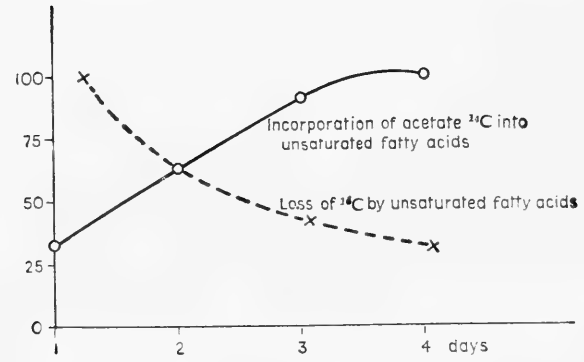

Fig. 2. Rate of incorporation of acetate ^{14}C (Pihl *et al.*) and rate of loss of ^{14}C by unsaturated fatty acids extracted from rat liver.

lioration of purification or measuring methods would hardly lead to more accurate mean renewal times, such could be obtained only by investigating an appreciably larger number of animals.

The significance of the data obtained is restricted, as both the saturated and unsaturated fatty acids represent a mixture of components having different turnover rates, some components of the unsaturated fatty acid mixture as linoleic or linoleic acid are not synthesized in the animal organism, and thus are not labelled. While Pihl and Bloch [8] state that the linoleic acid content of the rat liver is almost negligable, these authors find neutral fatty acids to contain 16 per cent, phosphatide fatty acids and 8 per cent of linoleic acid. From the fatty acids extracted from the liver of rabbits Popjak and Beeckmans [10] found that when the acetate was given to the animals for 20 hr the specific

activity of phosphatide fatty acids was larger than that of the glyceride fatty acids, in experiments of longer duration, however, no significant difference in the ^{14}C content of the two types of fatty acids could be observed.

In all our experiments 1 to 4 days after injecting the labelled acetate the specific activity of the total fatty acids of neutral fat of the rat liver was found to be a few per cent higher than the specific activity of phosphatide fatty acids, while according to PIHL and BLOCH (8) 3 days after feeding labelled acetate to rats kept on lipid free diet, the phosphatide fatty acids are 6 to 13 per cent more active than the total fatty acids of neutral fat.

As mentioned above, investigation of the incorporation of ^{14}C into fatty acids of the liver shortly after the administration of labelled acetate to mice revealed the existence of a *rapidly metabolising fatty acid fraction*. A rapid decrease in the ^{14}C content of total fat, neutral and phosphatide fatty acids of the liver of the rat is also observed shortly after the administration of labelled acetate but is less pronounced than in the case of the mouse liver. Some results obtained when injecting several rats each of which being killed at a different time after intraperitoneal injection of the labelled acetate are shown in Tables I*a* and *b*.

These and similar results are to be interpreted cautiously, among others because — in contrast to the experiments on mice where pooled livers of a large number of mice where extracted – in the present investigation each point indicates the result obtained when extracting a single liver only. The fatty acid metabolism may strongly vary from animal to

TABLE 1. — PERCENTAGE INJECTED ACETATE ^{14}C PRESENT IN 1 mgm OF NEUTRAL TOTAL FATTY ACIDS OF NEUTRAL FAT EXTRACTED FROM THE LIVER OF 182—190 gm RATS

Time after injection	Percentage injected × 10^3 in 1 mgm total fatty acids of neutral fat
	a)
20 min	37.5
80 „	51.4
110 „	63.0
140 „	21.1
170 „	23.2
240 „	28.3
	b)
20 „	56.2
50 „	66.0
80 „	79.2
110 „	38.0

animal, even when experimenting with animals of the same race, age and weight. The reason of these variations is inherent partly in the constitution of the livers compared, and is presumably partly the result of a difference in the amount and time of food taken in by the rat. A difference of minutes only in the time when the animal last took in food may influence the renewal rate of the fatty acids of the liver.

In spite of the great variations in the rate of ^{14}C incorporation into liver fatty acids the results shown in Table 1 and further similar results indicate a more rapid loss of fatty acid ^{14}C in the first hours of the experiment than in the later phase, the maximum ^{14}C incorporation being observed 1—2 hr after injecting the acetate. One may be inclined to interpret the rapid decrease in the ^{14}C content of the fatty acids during the first hours of the experiment as a consequence of the presence of an impurity in the fatty acid fraction. After purification of the fatty acids or the phosphatides according to FOLCH and VAN SLYKE's method (3) the decrease was still observable. For the fatty acids of the mouse liver the ^{14}C content of the isolated lead salts has furthermore shown a similar time dependency to that of fatty acid mixture (2). LEHNINGER's copper-calcium method of purification, while increasing the activity figures with 10—20 per cent through removal of less active fractions, did not influence significantly the time dependency of activity figures obtained. The same applies to the already mentioned purification with colloidal iron hydroxyde. The specific activity of phosphatide fatty acids, for example, purified by this method, was increased up to 40 per cent ; however, the percentage change in ^{14}C content of the fatty acids secured at different times after injecting labelled acetate to the rat was not significantly influenced by the purification process.

Summary

Since most of the ^{14}C injected as acetate into the rat is exhaled within a day, incorporation of ^{14}C into fatty acids of the liver after that date can be neglected in the first approximation. From the rate of loss of ^{14}C by the fatty acids of the liver of rats, to which one or more days previously labelled acetate was administered, the rate of renewal of the fatty acids can thus be calculated. The half-life of the saturated fatty acid mixture was found to be 0.8 days, that of the unsaturated fatty acid mixture (1 to 4 days after injection of the labelled acetate) to amount to 2.2 days.

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Comment on papers 41—43

In some of their earliest classical investigations, Schoenheimer and Rittenberg using deuterium as an indicator found the half-life of fatty acids of the liver of the rat to be from 1 to 2 days. Investigating with Dreyfus the effect of irradiation on the turnover rate of the fatty acids of the liver of mice very shortly after administration of ^{14}C labelled acetate (paper 81), we found the presence in the liver of a fatty acid fraction having the half-life of some minutes only. No such fraction was found in the fatty acids extracted from the brain or the muscles (paper 39). Beeckmans and Elliot (1951) succeeded in our laboratory in demonstrating that both the saturated and unsaturated fatty acids of the liver contain a short-living fraction, and Swan (1951) could show that we are not faced with only a re-carboxylation of the molecule, but with a renewal of all its carbon atoms. Recently, a short-living fatty acid fraction was found also in the liver of the rat (Tove 1956).

The half-life of molecules is usually determined by following the incorporation of the tracer into the newly formed molecules. As described in paper 41 we can also determine the half-life of the liver fatty acids by labelling these and following the rate of decrease in their activity.

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42. RATE OF PENETRATION OF IONS THROUGH THE CAPILLARY WALL

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In this paper, the results of experiments are communicated which were carried out in order to get information on the rate of passage of the ions of important constituents of the plasma as sodium, potassium, chlorine, and phosphate through the capillary wall. Crystalline substances introduced into the circulation will soon invade the extracellular fluid of the body. On this fact is based the method usually applied to determine the size of the extracellular space. Sucrose, sulphocyanate, or sulphate introduced into the human circulation were found (Lavietes *et al.*, 1936), for example, to be completely distributed between the plasma and the tissue space in the course of two or three hours. A complete distribution of thiocyanate in the extracellular space of rabbits in the course of half an hour is recorded (Krogh, 1937).

The partition of a substance introduced into the circulation between plasma and the extracellular fluid involves two processes : (1) penetration across the capillary wall and (2) distribution by diffusion and convective processes in the capillary and the extracellular fluids. The intrusion into the capillaries will play a secondary role, only, in view of the very short distances between the capillaries. Taking the length of the distances involved (Krogh, 1926) to be less than 60 μ and the diffusion coefficient of the substance investigated to be at least 1 cm^2 per day, the time necessary to displace, for example, a sodium ion from one end of the capillary space to the other, or from one end of the corresponding extracellular space to the other, will be less than 2 sec[1]. We arrive at this result by considering the propagation by diffusion only of the substance which penetrated the capillary wall. The fluid is, however, not without a circulation of its own, and this circulation will possibly shorten the time arrived at in the above calculation.

By introducing some sodium chloride into the circulation and by measuring the time it takes for a certain fraction to leave the circulation,

[1] The mean displacement of a particle $\tau = \sqrt{2\ D}$, where D is the diffusion coefficient.

it should be possible to measure the rate of passage of sodium chloride through the endothelium. However, when carrying out these experiments we meet the following difficulties : (a) Not only does the circulation get rid of excess sodium chloride by giving off salt to the extracellular space, but also by taking water up from the tissues. KEYES (1937) found, when studying the fate of sucrose intravenously injected into man, that osmotic equilibrium by a shift of water takes place from three to ten times as fast as sucrose exchange. The rate of disappearance of the excess sodium chloride will, thus, not measure the rate of passage of sodium chloride through the capillary wall but a more complex process. (b). The resistance of the endothelium to the passage of sodium and chloride may be quite different. (c) The introduction of appreciable amounts of sodium chloride into the circulation will disturb the normal conditions prevailing in the circulation. When one tries to eliminate this difficulty by introducing small amounts only, the analytical difficulties become almost unsurmountable. All these difficulties can be eliminated by injecting into the veins labelled sodium chloride (sodium chloride containing some radioactive ^{24}Na of negligible weight) and by measuring the rate of disappearance of the active ions from the plasma, i. e. the decrease of the radioactivity of the plasma. We are not determining in these experiments the rate of influx of excess sodium chloride from the plasma into the extracellular fluid but the rate of exchange between labelled plasma sodium and non-labelled extracellular sodium, as the number of sodium ($^{23}Na + ^{24}Na$) atoms of the plasma remains practically constant all through the experiment. The rate of exchange will be determined by the permeability of the capillary wall to sodium ions and will, thus, be a measure of this permeability.

We carried out also experiments with radio-potassium, radio-chlorine, radio-bromine, and radio-phosphate, while heavy water was used as an indicator for water in the study of permeability of the endothelium to water. The measurement of the distribution of radio-sodium between plasma and the extravascular space of the rabbit was previously used to determine the extracellular volume of the rabbit (GRIFFITH and MARGRAITH, 1939 ; HAHN *et al.*, 1939).

EXPERIMENTAL PROCEDURE

Radioactive sodium and potassium were prepared by bombarding NaOH and KOH, respectively, with high speed (10 million volts) deuterons. The hydroxydes were neutralized with hydrochloric acid and the solution thus obtained was injected. Radioactive chlorine and bromine were prepared by bombarding NaCl and NaBr, respectively, with deuterons. The active chlorine and bromine obtained were driven off as HCl and HBr, respectively, and were collected in a sodium hydroxyde solution. This procedure was chosen to get rid of the active sodium simultaneously

produced with the active halogens. We are much indebted to Dr. J. C. JACOBSEN and Mr. O. N. LASSEN for preparing the radioactive substances by making use of the Copenhagen cyclotron.

About 3 cc. solution containing the radioactive substances of an activity of about 1 microcurie was applied. The salt concentration of these solutions was

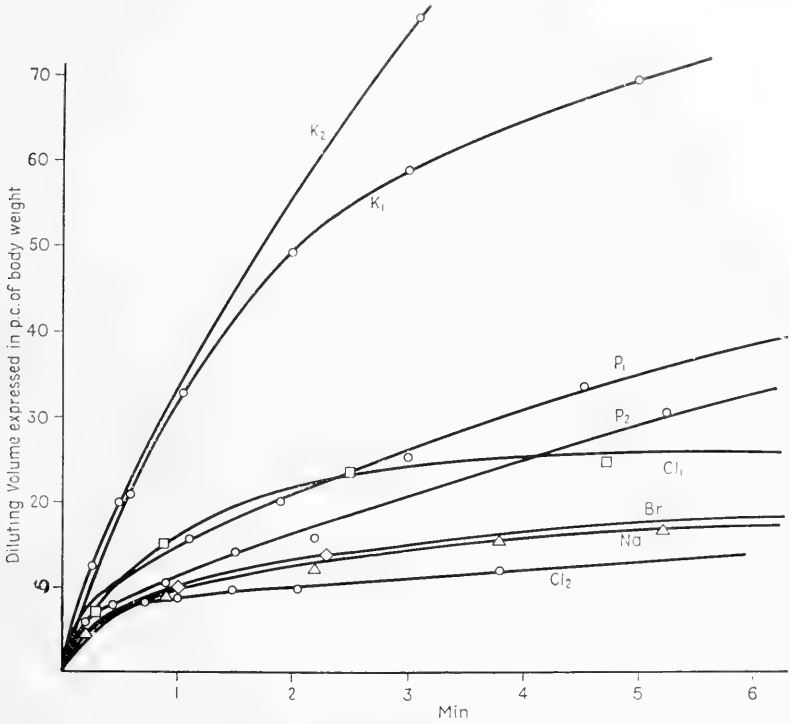

FIG. 1. Rate of disappearance of various labelled ions from the plasma.

brought up to a physiological level by adding non-active sodium chloride. The solution was injected into the jugularis of the rabbit and blood samples of about 1 cc. were collected at intervals from the carotis. Plasma samples of known weight were dried and their radioactivity was compared by using a Geiger counter. For comparison of the radioactivity of plasma and muscle samples the samples were ashed at about 400° and the plasma ash mixed with non-active muscle ash of the same weight as the corresponding active muscle ash sample. Blood and muscle samples were secured simultaneously from the narcotized rabbit.

RESULTS

The results obtained are seen in Tables 1 to 5 and Figs. 1 to 3. The tables contain data on the percentage of the labelled element injected still present in 1 cc. plasma at various intervals. The volume of diluting fluid necessary to bring down the concentration of the substance in-

jected to that found after a given time is also stated. Furthermore, the diluting volume is expressed in percent of the rabbit's body weight.

We shall first compare the rate of disappearance of sodium, chlorine, and bromine from the circulation. This comparison encounters no difficulties since practically the sole outlet of these elements from the circulation is the extracellular body fluid, though some ^{24}Na is taken up by the bone apatite (Hahn *et al.* 1939). No great diffe-

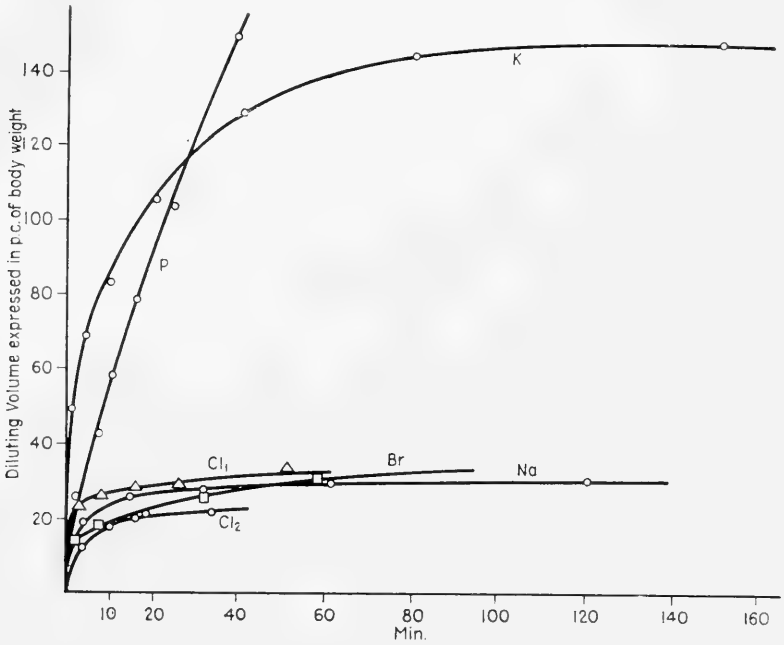

Fig. 2. Rate of disappearance of various labelled ions from the plasma.

rence is found between the rate of passage of sodium, chlorine, and bromine through the capillary wall but the values obtained for different rabbits show fairly large variations. These variations are to some extent due to differences in the size of the extracellular space.

A comparison of the rate of passage of potassium, phosphate, and water with that of sodium, chlorine, and bromine encounters some difficulties since potassium, and the same applies to phosphate and water, has an additional outlet into the tissue cells in contrast to the first mentioned group. The amount of ^{42}K lost by the blood after the lapse of a given time is the resultant of the amount penetrated into the tissue fluids and that returned from the latter into the blood. When

[1] In experiments taking up to 1 hour, the amount of ^{24}Na lost by excretion is less than 1 per cent of the amount administered.

TABLE. 1. — RATE OF DISAPPEARANCE OF ^{38}Cl FROM THE CIRCULATION OF RABBITS WEIGHING 2.5 AND 2.4 KGM, RESPECTIVELY

Time in min	Percent of ^{38}Cl injected present in 1 gm plasma	Diluting fluid volume	
		in cc. (apparent extracellular volume)	in percent of body weight
	Rabbit I.		
0.37	0.622	161	6.4
0.73	0.486	206	8.2
1.01	0.475	211	8.5
1.48	0.408	245	9.8
2.05	0.400	250	10.0
3.8	0.329	304	12.2
10.5	0.224	446	17.8
18.5	0.188	532	21.3
35	0.182	550	22.0
	Rabbit II.		
0.3	0.62	161	6.7
0.9	0.28	357	14.9
2.5	0.174	575	24.0
4.7	0.165	607	25.3
8.3	0.161	622	26.0
16.5	0.150	668	27.8
26	0.143	700	29.2
51	0.128	783	32.6

besides the interspaces the cellular space opens an outlet to the ^{42}K leaving the circulation, the amount returning from the tissue fluids into the blood will be reduced and, thus, the resultant ^{42}K concentration of the plasma will be lowered. Though the potassium content of the cells is only partly replaced by ^{42}K during the experiment in view of

TABLE 2. — RATE OF THE DISAPPEARANCE OF ^{80}Br FROM THE CIRCULATION OF A RABBIT WEIGHING 2.7 KGM

Time in min	Percent of ^{80}Br injected present in 1 gm plasma	Diluting fluid volume	
		in cc. (apparent extracellular volume)	in percent of body weight
1.0	0.37	270	10.0
2.2	0.27	370	13.7
8.1	0.21	475	17.6
16.3	0.18	556	20.6
32	0.14	715	26.5
58.5	0.12	835	30.9

the low potassium content of the plasma and the high content of the tissue cells, the additional outlet opened by the intrusion of ^{42}K into the cells in experiments taking one hour, makes out about five times the normal outlet of intrusion of these ions into the interspaces.

The total water content of the cells can be entirely replaced by labelled water. Since the volume of the cellular body water is about twice as large as that of the extracellular fluid through the intrusion of labelled water into the cells a substantial additional outlet of the labelled water molecules of the plasma is opened.

TABLE 3. — RATE OF DISAPPEARANCE OF ^{24}Na FROM THE CIRCULATION OF RABBITS WEIGHING 2.7 KGM

Time in min	Percent of ^{24}Na injected present in 1 gm plasma	Diluting fluid volume	
		in cc. (apparent extracellular volume)	in percent of body weight
	Rabbit I.		
0.2	0.80	125	4.6
0.45	0.50	199	7.4
0.9	0.41	242	9.0
1.5	0.32	310	11.5
2.2	0.30	331	12.3
3.8	0.234	427	15.8
5.2	0.215	466	17.3
11	0.194	515	19.1
	Rabbit II.		
1.3	0.63	158	6.6
2.3	0.282	357	14.9
4.3	0.218	458	19.1
9.1	0.181	553	23.0
15	0.163	615	25.6
32	0.151	663	27.6
61	0.140	715	29.8
120	0.134	745	31.1

As to the phosphate ions, not only that they diffuse into the tissue cells but they are also incorporated into the the bone apatite. These additional outlets may be made responsible for the fact that the ^{32}P loss of the plasma is found to be very much greater than the ^{24}Na loss during the same time.

While the additional outlets mentioned above for potassium, phosphate, and water will be responsible for the high values of the volume of the diluting body fluid observed for these elements in experiments of comparatively long duration, the fact that also after the lapse of $\frac{1}{4}$ min

TABLE 4. — RATE OF DISAPPEARANCE OF ^{42}K FROM THE CIRCULATION OF RABBITS WEIGHING 2.5, 2.4 AND 2.3 KGM, RESPECTIVELY

Time in min	Percent of ^{42}K injected present in 1 gm plasma	Diluting fluid volume	
		in cc. (apparent extracellular volume)	in percent of body weight
	Rabbit I.		
0.6	0.19	526	21
2.0	0.082	1220	49
3	0.068	1470	59
5	0.058	1720	69
15	0.035	2860	114
	Rabbit II.		
5	0.058	1730	72
10.5	0.048	2080	87
20.5	0.038	2640	110
40.5	0.031	3220	134
80	0.0277	3610	151
210	0.0269	3720	155
	Rabbit III.		
0.26	0.354	283	12.3
0.50	0.220	455	19.8
1.05	0.129	775	33.7
3.05	0.057	1755	76.3

only, larger amounts of potassium than of sodium, chlorine, or bromine are lost by the plasma, requires possibly another explanation. After such a short time the volume of the diluting fluid is much smaller than the extracellular space of the rabbit and the additional outlet can possibly not play a decisive role. The very rapid disappearance of potassium from the circulation suggests the assumption that potassium, when passing the endothelium, encounters appreciably less resistance than does sodium or chlorine. The diffusion constant of potassium in water is larger than that of sodium ; taking the former to be 1, the diffusion constant of sodium makes out 0.65. The diffusion constants of potassium and chlorine are practically identical. The rates of penetration of potassium and chlorine through the endothelium, however, differ greatly. The diffusion rate for water in water was found, using heavy water as an indicator, to be only 1.6 times larger, than that of chlorine or potassium, while the rate of passage of water through the endothelium is very much faster than that of any other substance investigated by us. In the course of 21 sec the labelled water introduced into the circulation of the rabbit is found to be distributed in 506 cc. body water, corresponding to 34 percent of the rabbit's weight.

TABLE 5. — RATE OF DISAPPEARANCE OF ^{32}P FROM THE CIRCULATION OF RABBITS WEIGHING 2.1 AND 2.7 KGM., RESPECTIVELY

Time in min	Percent of ^{32}P injected present in 1 gm plasma	Diluting fluid volume	
		in cc. (apparent extracellular volume)	in percent of body weight
	Rabbit I.		
1.1	0.300	333	15.9
1.9	0.234	427	20.4
3.0	0.187	535	25.5
4.5	0.143	699	33.3
6.8	0.112	892	42.5
10.9	0.081	1230	58.6
16.9	0.060	1670	79.5
25.9	0.046	2180	104
39.0	0.032	3130	149
	Rabbit II.		
0.2	0.63	160	5.9
0.45	0.46	216	8.0
0.9	0.35	287	10.6
1.5	0.261	383	14.2
2.2	0.234	428	15.9
5.2	0.120	835	30.9

When investigating the staining capacity of dyes it was found that generally the rate of coloration increases with decreasing diffusion rate in water (ROUS *et al.* 1930 ; SMITH and ROUS 1931 ; MENKIN and MENKIN 1930). From the capillaries of the frogs mesentery trypan blue was found to disappear exponentially with a half-time period of 2 min (MENKIN and MENKIN 1939).

We have not yet mentioned the fact that an outlet of the plasma ^{24}Na, for example, is given by intrusion into the corpuscles. This outlet is a very restricted one. We found the ^{24}Na content of 1 gm corpuscles of the rabbit to be, after the lapse of two hours, 11 percent of that of 1 gm plasma. From this figure and the haematocrit value (34 percent) of rabbit blood it follows that, expressed in diluting body fluid volume, the uptake of ^{24}Na by the corpuscles corresponds to somewhat less than 4 cc., while the total diluting volume of a rabbit weighing 2,5 kgm makes out as much as about 700 cc. After the lapse of $1\frac{1}{2}$ hours, the ^{42}K content of 1 gm corpuscles was found to be 40 percent of that of 1 gm plasma and the ^{32}P content 48 percent. These figures correspond to an additional diluting volume of 14 and 16 cc., respectively. The water content of the corpuscles of the rabbit being about 63 percent, the role of the corpuscles as additional outlet of the labelled water molecules introduced into the plasma is not quite insignificant.

Permeability of Muscle and Brain Capillaries

The figures stated in the preceding section give information on the rate at which ions and molecules leave the capillary system. Should a minor part of the capillary wall be slightly permeable or even impermeable to some of the substances investigated, this would not have been revealed by the figures given above, since these figures indicate the permeability of the very inhomogeneous capillary system *in toto*. If we want to know the average permeability of the muscle capillaries, for example, to ^{24}Na, we have to compare the ^{24}Na content of plasma and muscle samples of known weight.

The results of such measurements are seen in Table 6, in which the percentage ratio of the ^{24}Na content of 1 gm fresh gastrocnemius tissue to 1 gm plasma is given. The table contains also data on the ^{32}P content of muscles and the ^{24}Na and ^{32}P content of the brain tissue. After the lapse of 11 min, a proportional partition of ^{24}Na between plasma and

TABLE 6. — RATIO OF THE ^{24}Na AND ^{32}P CONTENT, RESPECTIVELY, OF 1 GM TISSUE AND 1 GM PLASMA

Tissue	Time in min	Ratio of the content of 1 gm tissue and 1 gm plasma × 100	
		^{24}Na	^{32}P
Muscle	0.9	3.74	0.98
Muscle	5.2	7.75	3.42
Muscle	11	10.7	8.82
Muscle (other rabbit)	120	11.5	—
Brain, total	11	3.0	4.5
Brain, white	11	2.2	—
Brain, grey	11	3.9	—
Brain, white (other rabbit)	120	10.9	—
Brain, grey (other rabbit)	120	14.9	—
Medulla oblongata (other rabbit)	120	17.2	—

gastrocnemius is nearly reached, since the size of the extracellular space of the gastrocnemius of the rabbit is about 11 per cent of the weight of the muscles. The muscle capillaries are seen to be more permeable to sodium than to phosphate : in the course of the first minute about four times more ^{24}Na left the plasma for the muscle tissue than ^{32}P.

The permeability of the brain capillaries to ^{24}Na and also to ^{32}P is lower than that of the muscle capillaries, those of the white brain substance being apparently less permeable than those of the grey brain substance to ^{24}Na[1]. After the lapse of 62 hours, we found (HAHN *et al.*

[1] This is possibly an expression of the fact that the vascularity of the grey matter greatly exceeds that of the white.

1939) the ^{24}Na content of 1 gm brain to make out 32 percent of that of 1 gm plasma, a figure which corresponds to that stated (MANERY and HASTINGS, 1939) for the sodium space of the brain.

MANERY and BALE (1939) carried out experiments with labelled sodium and phosphorus. They state that, in the course of an hour, the ^{24}Na uptake by the brain and the sciatic nerve is much smaller than to be expected in the case of a proportional partition of ^{24}Na between plasma and interspaces of these organs while, in the case of the other organs, such a partition was reached after the lapse of 20 min. WALLACE and BRODIE (1937,1939) investigated the distribution of iodide, thiocyanate and chloride in various tissues of the body and found that the relative concentration in terms of blood tissue ration was alike for the three substances in the organs examined, with the exception of the brain in which the chloride was in much larger amounts that the other two. In a later investigation (1939) these authors found that these anions distribute in the central nervous system in the same ratio to chloride as in spinal fluid, whereas in other body tissue they distribute in the same ratio to chloride as in serum. That sulphate passes more slowly than bromide or nitrate through the brain capillaries can be concluded from experiments in which the chloride content of the plasma was replaced by sulphate (AMBERSON *et al.* 1938), bromide (WEIR 1936), and nitrate (HIALT 1939), respectively.

We compared, furthermore, the activity of 1 gm of the grey brain substance with that of 1 gm plasma 59 min after the administration of radiobromide. The ratio found was 9.3 per cent. As the chlorine space of the brain tissue of the rabbit is 35 per cent, we have to follow that in the course of an hour, less than $^1/_3$ of the proportional partition of bromine between plasma and the extracellular volume of the brain is obtained.

Permeability to Water

We investigated previously the rate at which heavy water introduced into the circulation leaves the plasma (HEVESY and JACOBSEN, 1940). These measurements were extended by determining the distribution of labelled water between plasma and gastrocnemius of equal weight. The figures obtained are seen in Table 7. The water content of the samples was driven off and was collected *in vacuo* as described previously. We are much indebted to Miss HILDE LEVI for kindly determining the density of the water samples obtained by making use of LINDERSTRÖM-LANG's floating drop method.

The density excess of these water samples over the density of normal body water is a measure of their labelled water content. The ratio of the density excess of plasma water and muscle water is stated in Table 7 and Figure 3.

A ratio equal to 1 is to be expected when the heavy water concentration of the total muscle water corresponds to that of the plasma water. This stage of partition is reached between 20 and 38 min. after the start of the experiment[1]. This is the same result at which HEVESY and JACOBSEN

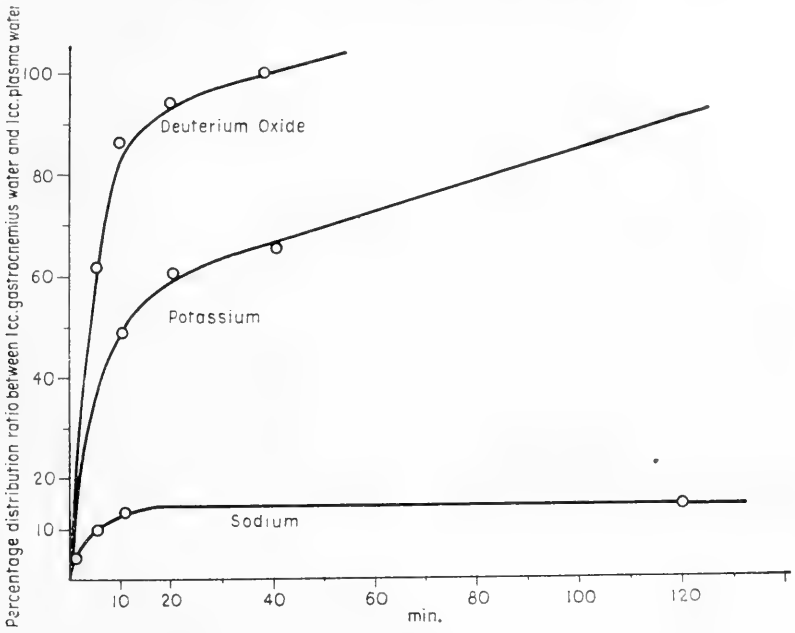

FIG. 3. Percentage distribution ratio of labelled sodium, potassium and deuterium oxyde between plasma water and muscle water of equal weight.

TABLE 7. — RATIO OF THE DENSITY EXCESS OF PLASMA WATER AND MUSCLE WATER

Time in min	Ratio of density excess of plasma water and muscle water
5	1.62
10	1.16
20	1.06
38	1.00

(1940) arrived when investigating the rate of disappearance of heavy water from the circulation. The water content of the gastrocnemius makes out 77 per cent of the muscles weight, about 11 per cent being

[1] Though great care was taken to distill off the total water content of the muscle, the possibility that a minor amount of muscle water was not removed can not be excluded. This water may not have taken part in an exchange process.

located in the interspaces and the rest in the cells. If the extracellular water should alone take part in the exchange process, we should expect in the case of a proportional partition of the heavy water between plasma and extracellular water the ratio of the density excess to be about 7. From the fact that this ratio is found after lapse of 5 min. to be but 1.62 we have to conclude that during that time not only a proportional partition of the labelled water between plasma and the extracellular fluid of the muscles took place, but a large part of the cell water was replaced by plasma water as well.

Summary

Solutions of labelled chloride, bromide, sodium, potassium, phosphate and deuterium oxyde were injected into the circulation of rabbits and the speed of the escape of the labelled ions from the plasma was determined. Labelled potassium was found to leave the circulation at a faster rate than any other ion investigated.

Information on the permeability of the muscle and brain capillaries were obtained by comparing the labelled sodium, phosphate and heavy water content of muscle and plasma, respectively brain and plasma. The muscle endothelium was found to be more permeable to sodium and to phosphate than the endothelium of the brain. The partition ratio of labelled sodium between plasma and the chloride space of the brain amounts after the lapse of 11 min only to $^1/_{30}$ of the equilibrium value; during that time $^1/_{10}$ of a proportional partition of labelled sodium between plasma and the chloride space of the muscle was obtained.

In the course of an hour, somewhat less than $^1/_3$ of the proportional partition of bromine between plasma and the grey brain substance was reached. Proportional partition of labelled water between plasma and the muscle water was observed after about half an hour.

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43. RATE OF PASSAGE OF WATER THROUGH CAPILLARY AND CELL WALLS

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Water molecules, which are absorbed into the circulation, will mix rapidly with those present in the plasma. They will then penetrate the capillary wall and become distributed in the extracellular fluid. Ultimately, they will invade the cells. Simultaneously, a loss of some of the water molecules through the kidneys, the bowels, the lungs and through peripheral evaporation will also take place. It is difficult to estimate even very roughly the rate at which some of the above processes take place. Experiments in which heavy water is used as an indicator permit, however, the determination of the rate at which individual water molecules introduced into the circulation are distributed in the body water and from these determinations to answer the above questions.

We inject a few cc. of practically pure heavy water into the jugularis of a rabbit and take at intervals blood samples from the carotis. The next step is to prepare pure water from the blood samples[1] and to determine ist density. Let us assume that we inject 1 cc. of heavy water having a density of 1.1000, and find for the water prepared from a blood sample the value 1.001. Then we must conclude that the 1 cc. heavy water injected into the vein was diluted by 99 cc. of normal water present in the body of the rabbit in the course of the time which elapsed between the injection of the heavy water and the collection of the blood sample.

EXPERIMENTAL PROCEDURE

Heavy water in contact with air containing vapour of normal water rapidly becomes lighter in consequence of the interchange in the vapour phase. In view of this interchange, it was necessary to keep the samples containing heavy water out of contact with the moist atmosphere. The blood samples of about 1 cc.

[1] The total water content of the blood has to be distilled to avoid a fractionation of the diluted heavy water, the vapour pressure of deuterium oxyde being smaller than that of H_2O.

volume were collected in small dishes containing traces of heparin, the blood was transferred into part A of the glass vessel, seen in Fig. 1. and the vessel closed with soft paraffin. These operations took only a few seconds. About ½ mgm of dry P_2O_5 was added to the solution in order to neutralize any traces of ammonia present. The vessel was then cooled in the refrigerator, evacuated, and sealed off at B. Tube D was then immersed in liquid air, while the other parts of the vessel remained at room temperature. After the lapse of a few hours, the water content of the blood sample was found to be present in the form of ice in tube C. After the ice was molten and the water collected in D, this tube was sealed off. The pure water obtained by this procedure was further purified by distillation in the presence of potassium permanganate and sodium peroxyde, and the density of the purified samples determined, using Linderström—Lang's floating drop method (Linderström—Lang, Jacobsen and Johansen, 1938).

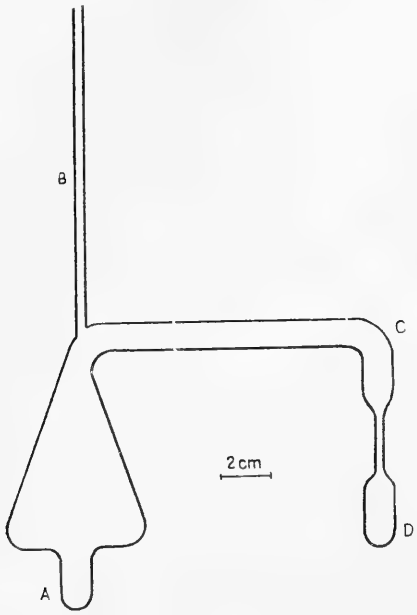

Fig. 1.

Experiment A

In this experiment, a rabbit weighing 2.6 kgm was used. 9.2 cc. of heavy water having a density of 1.1049 were injected. The time which elapsed after the injection of the heavy water is recorded in the first column of Table 1, while the next column contains data on the density excess of the blood water over normal water, expressed in parts per million. (The heavy water injected had a density excess of 104900 parts per million.) The third column contains data on the dilution of 1 cc. heavy water introduced into the circulation. In the fourth column the percentage of the weight of the rabbit which took part in the dilution process is stated.

The injection lasted 40 sec.; the time recorded in column 1 is calculated from the moment half of the water was injected; the first blood sample was collected

Table 1

Time	Density excess of blood water in parts per million	Extent of dilution of 1 cc. heavy water	Diluting water volume expressed in percentage of body weight
40 sec.	1 500	644	24.7
185 sec.	1 300	742	28.6
3.3 min. ..	930	1 038	40.0
6.1 min. ..	795	1 221	46.9
24.1 min. ..	527	1 825	70.2
5 days ..	339	2 843	109
39 days ..	89	10 810	417

20 sec. after all the heavy water was injected. In the course of such a short time as 40 sec., 644 cc. of the body water took part in diluting the heavy water injected and, after the lapse of 24 min., as much as 1825 cc.

The plasma water cannot diffuse into the cells without passing the capillary wall. If the last mentioned process took place within 40 sec., then the volume of the diluting water should be at least equal to that of the extracellular fluid which amounts, in a rabbit weighing 2.6 kgm, to 670 cc[1] As seen in column 4, the volume of the diluting water was only slightly less, namely 650 cc., than this value. The total water content of the rabbit amounts to 70 to 75 per cent of its weight, corresponding to a volume of 1820 to 1950 cc. As seen in column 4, the sample collected after the lapse of 24 min was found to be diluted by 1825 cc. body water. Within that time, therefore, a distribution of the heavy water in almost the total body water took place, though some of the water present in certain organs may not have taken part in the exchange process[2] This point can only be settled by investigating the density of tissue water. The above figures suggest that, in contradistinction to a very fast invasion of the interspaces, the penetration into the cells is a somewhat slower process.

In the samples collected shortly after the start of the experiment the dilution, due to a loss of heavy water by the body, can be disregarded. This is not the case in experiments lasting several hours or days. In the course of 5 days, for example, the loss of water through the kidneys alone amounts to about 1 liter, thus to 38 per cent of the rabbit's weight. In this case, the excretion of a corresponding part of the heavy water is responsible for the, at first sight puzzling, value of 109 per cent found. Some of the hydrogen atoms bound to oxygen or nitrogen in the various organic compounds present in the body, exchange with those present in the water or heavy water molecules, and this process will also increase the dilution figures observed, as a removal of deuterium acts in the same way on the water density figures as does dilution by normal water. In view, however, of the fact that the amount of hydrogen present in the organic compounds is small compared with that of the hydrogen incorporated in water molecules, the process mentioned above will not much influence the dilution figures obtained. In experiments of long duration, a successive replacement of most of the hydrogen atoms present in organic molecules will take place, giving an additional outlet to some of the deuterium atoms present in the body water. The percentage of hydrogen present in the fats of the body which exchanges within 1 hour with water hydrogen is negligible; the corresponding amount of protein hydrogen is not (Ussing, 1938). The water equivalent of this hydrogen amounts, however, only to ½ to 2 per cent of the body weight, or 13 to 52 cc. in the case of rabbit A and 8 to 30 cc. in the case of rabbit B. The amount of catabolic water formed in the course of 1 hour in the rabbits amounts only to about 0.1 per cent of the body weight. As seen in Table 1, after the lapse of 39 days, the density excess of the blood water very much declined. This decline is mainly due to loss of the heavy water and, thus, of a corresponding amount of normal water present at the start of the experiment in the body. About 5/6 of these molecules was lost in the course of 39 days. In the case of human subjects, who drank heavy water, it was found (Hevesy and Hofer, 1934) that, in the course of 9 days, half of the heavy water taken was lost.

In the above connection, it is of interest to recall the experiments carried out by McDougall, Verzar, Erlenmeyer and Gaertner (1394). They injected

[1] Comp., for example, A. Krogh (1937).
[2] Comp. Ussing (1938).

a solution of heavy water into the jejunal loops of rats and investigated the heavy water content of the intestinal fluid of the rats killed 1 hour after the start of the experiment. They found the diluting water volume of the rat to amount to 66 per cent of the body weight.

Experiment B

This experiment was carried out on a rabbit weighing 1.5 kgm 5.0 cc. of heavy water were injected. The injection took 6 sec. The first blood sample was taken in the interval of 22—26 sec. after the start of the experiment. The time recorded is reckoned from the moment that half of the heavy water was injected until half of the blood sample was collected.

TABLE 2

Time	Density excess of blood water in parts per million	Extent of dilution of 1 cc. heavy water in the circulation	Diluting water volume expressed in percentage of body weight
21 sec. ...	1 034	506	34
80 sec. ...	857	612	40.8
1.8 min. ...	794	661	44.1
3 min. ...	719	727	47.8
5.2 min. ...	570	921	61.4
7.9 min. ...	495	1 056	70.4
13 min. ...	490	1 070	71.3
22.2 min. ...	440	1 190	79.4
30 min. ...	450	1 167	77.9
48 hours ..	412	1 274	85

While rabbit A had not shown any sign of distress after the blood samples were taken, this was not the case with rabbit B. In the course of the two days following the start of the experiment, only a small amount of urine was produced by rabbit B, with the consequence that no pronounced effect due to loss of heavy water through the kidneys is shown by the density figures of the blood water sample of rabbit B, collected after the lapse of 2 days, in contradistinction to the results obtained when investigating the blood water of rabbit A after the lapse of some days.

DISCUSSION

We found that, within about ½ min, heavy water injected into the jugularis of rabbits was diluted by a large amount of body water, the volume of which corresponds to about that of the extracellular space of the body. This rapid dilution is followed by a second, slower process, presumably due mainly to a further dilution of the heavy water by cellular body water. From these findings it follows that all water molecules present in the plasma pass with a very high speed through the capillary walls and with a slower, but still remarkable speed through

the cell walls, and vice versa. To this conclusion one may possibly object that heavy water (D_2O) may show a different behaviour from H_2O and recall the results obtained in the investigation of the rate of hemolysis of erytrocytes of cattle and rats, which was found to take place about 44 per cent more slowly in D_2O than in H_2O (PARPART, 1935 ; BROOKS, 1935).

Contrary to those of the above mentioned authors, our experiments were not carried out with pure D_2O but with very diluted heavy water, the viscosity and other properties of which only slightly differ from those of H_2O. Our most concentrated samples contained, in fact, less than 2 per cent D_2O, most of them containing very much less. The D_2O injected into the vein is diluted at once. The above mentioned authors did not use heavy water as an indicator for water ; they were interested in the differences shown by H_2O and D_2O when penetrating into corpuscles. When heavy water is used as an indicator, it should always be used in a state as diluted as possible, partly for the above reasons and partly because such diluted solutions contain mainly DHO which is very similar to H_2O, while D_2O is much less so.

In view of the high speed of capillary passage found in our experiments, it is of interest to calculate the time taken by the diffusion of water molecules through the capillary spaces. The mean displacement τ of water in water is $\sqrt{2\,D}$ (where D denotes the diffusion constant of water in water, determined by using heavy water as an indicator), is 2 cm per day (ORR and THOMSON, 1935). Taking the size of the capillary vessel as 20μ, we arrive at the result that the displacement of water molecules within that space takes 1×10^{-6} day, or about $^1/_{10}$ sec, thus an exceedingly short time.

Summary

Heavy water is injected into the vein of rabbits and blood samples taken at intervals from the artery. The density of the water prepared from the blood samples is determined and, from the density difference between the injected heavy water and the blood water, the extent of dilution, which the heavy water molecules experienced in the body at different times, calculated.

As soon as a ½ min after the injection, a dilution of the heavy water by an amount of body water corresponding in volume to about that of the extracellular space of the body, is found. This very rapid rate of dilution is followed by a somewhat slower dilution process, in which the cellular water participates.

After the lapse of less than ½ hour, the heavy water molecules are evenly distributed over almost the total body water.

After the lapse of 39 days, only about $^1/_5$ of the heavy water injected is still present in the body.

There is no reason to assume that the heavy water (mainly DHO) molecules show a markedly different behaviour from that of the normal water (H_2O) molecules present in the body, and we have, therefore, to conclude that within about

½ min. a sufficient flow of water takes place through the capillary walls to lead to an almost perfect mixing of the blood and the interspace water. An analogous interaction between cellular and extracellular water takes less than ½ hour.

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44. RATE OF PENETRATION OF PHOSPHATE INTO MUSCLE CELLS

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Certain constituents of the voluntary muscle are able to diffuse out of the muscle into a surrounding saline and can also diffuse into the muscle if previously dissolved in the saline in a sufficiently high concentration. There exists, in such cases, a given concentration of the substance in saline which will be in equilibrium with the tissue. This critical concentration provides a measure for the concentration of the substance in the tissue — or rather in such part of the tissue as is concerned in the diffusion. M. G. Eggleton (1933) carried out such experiments[1] in respect of phosphate exchange by immersing in Ringer's fluid an excised frog muscle at 2° for a few hours and found that in the resting muscle 20—30 per cent of the muscle water, corresponding to about 16—24 per cent of the weight of the fresh muscle, was involved in the diffusion system. Since 8—16 per cent of the weight of the gastrocnemius is composed of the interspaces into which the phosphate of Ringer's fluid will easily penetrate, the result mentioned above suggests either that phosphate can diffuse only into a certain fraction of the tissue beyond the extracellular volume or else that phosphate ions can penetrate only slowly into the muscle cells. That the latter alternative is more likely follows from the fact that no perceptible decrease in the total acid soluble phosphate content of muscles is apparent after fatigue, though a very perceptible increase in the inorganic P content of such muscles takes place. If the cell membranes were easily permeable to phosphate ions, a part of this excess phosphate should soon leak out into the plasma.

The application of the method of isotopic indicators permits us to follow the path of the labelled phosphate ions introduced into the circulation by making use of radioactive measurements. Due to the great sensitivity of this method it is possible to determine even very small amounts of labelled phosphate ions which migrate under strictly physiological conditions into the muscle cells during a few hours or less.

[1] Comp. also Stella (1928).

DESCRIPTION OF THE METHOD

Sodium phosphate of negligible weight containing radioactive P as an indicator is introduced into the circulation of the frog (injected into the lymph sack). After the lapse of, for example, ten hours, we compare the labelled phosphorus (^{32}P) content of a plasma sample and of a gastrocnemius sample of the same weight by determining their radioactivity. Let us assume that 1 gm plasma is found to be 5 times more active than 1 gm muscle tissue and the interspaces to make up $^1/_{10}$ of the muscle's weight, then the amount of phosphate ions which penetrated from 1 gm plasma into the cells of 1 gm muscle works out to be $^1/_{10}$ of that present in 1 gm plasma or, in general, is $\frac{m}{p} - i\ p$, where m denotes the activity of 1 gm muscle, p the activity of 1 gm plasma, and i the size of the interspaces as a fraction of the muscle weight. When carrying out the calculation given above, we assume that ^{32}P becomes equally distributed between plasma and interspaces in an early stage of the experiment. How far this assumption is justified will be discussed later.

The experiment described above is carried out under strictly physiological conditions ; the phosphorus content of the plasma and the muscle remains practically constant during the experiment and we can, therefore, conclude that the penetration of phosphate ions from the plasma into the muscle cells was followed by a migration of an equal number of phosphate ions from the muscle cells into the plasma. If an equilibrium is reached with an uptake of less ^{32}P than corresponding to the total water content of the system, we must assume saturation of a certain fraction of the tissue but, when the relative concentration of ^{32}P continuously increases in the muscle, we can utilize the results to measure the rate of exchange between cellular and extracellular phosphorus.

The application of the method outlined above requires the knowledge of the extent of the interspaces of the muscle tissue. The size of the interspaces can be obtained by comparing the chloride or sodium content of muscle and plasma samples of the same weight or by other methods. When applying the first mentioned method, the assumption is made that all sodium and chlorine present in the tissue is to be found in the interspaces. Fenn and Cobb (1935) state for the chlorine space of the sartorius of rana pipiens values varying between 7.5 and 16.0 per cent, the average being 11.3 per cent.

To determine the size of the extracellular space of the gastrocnemius of the Hungarian frog (Rana esculenta) used in our experiments, we administered labelled sodium along with the labelled phosphate. By measuring the distribution of the labelled sodium ^{24}Na between plasma and fresh gastrocnemius of equal weight, we arrive at a figure indicating

the extracellular volume of the muscle. The amount of extracellular phase (E) in percent of the weight of the muscle is calculated (MANERY and HASTINGS, 1939) from the equation

$$E = \frac{(^{24}Na)_m \cdot 0.97 \cdot 100}{(^{24}Na)_p \cdot 0.99}$$

in which the subscripts m and p represent muscle and plasma, respectively[1].

The size of the interspaces being known, the measurement of the distribution of ^{32}P between plasma and muscle permits us, as described above, to determine the amount of ^{32}P which penetrates into the muscle cells.

A simultaneous measurement of the radioactivity of sodium and phosphorus is made possible by the fact that ^{24}Na decays with a half-life period of 14.8 hours while ^{32}P decays with a period of 14.5 days. When measuring the activity of the sample, for example, two weeks after the start of the experiment, the ^{24}Na originally present in the tissue and the plasma is entirely decayed and the activity measured is solely due to the ^{32}P content. Let us say we measured at that date 10 counts per minute, then two weeks previously the activity of the ^{32}P of the preparation was 20 counts. Assuming we measured, two weeks previously, a total of 100 counts, then out of these 80 counts were due the ^{24}Na content and 20 counts to the ^{32}P content of the gastrocnemius sample. The accuracy of the determination can be augmented by administering a preparation which is showing a strong ^{24}Na and a comparatively weak ^{32}P activity. In view of the variability of the size of the interspaces in different muscles and in different frogs (comp. EGGLETON *et al.* 1937), it can be of importance to determine the extracellular volume of the muscle the permeability of which to phosphate ions is to be determined.

In experiments of short duration, the P activity of the plasma is solely due to the presence of radioactive inorganic P, the amount of radioactive phosphatides present being negligible and the plasma containing but an insignificant amount of acid soluble organic P. In experiments of very long duration, we are not permitted to consider the total activity of the plasma, but we have to extract the plasma inor-

[1] When carrying out the calculation mentioned above, we assume that the extracellular phase is identical with the ultrafiltrate of serum. The water content of the extracellular phase is assumed to be 99, that of the plasma 95 per cent. We arrive at the figure 0.97 by taking into consideration that the sodium ion concentration of the plasma and its ultrafiltrate somewhat differs and by calculating the difference from the Gibbs-Donnan equation.

ganic P and to compare its activity with the total activity of the muscle cells, assuming that only inorganic P can penetrate into the cells and is afterwards largely combined in the cells.

RESULTS

The distribution of ^{24}Na and of ^{32}P between equal weights of plasma and gastrocnemius muscle is seen in Table 1. The ^{24}Na was administered as 0.6 per cent sodium chloride solution which contained a negligible amount of active sodium phosphate. The solution was injected into the lymph sack of the frog. While, in experiments taking no more than two hours, the phosphate solution was administered at the start of the experiment, in experiments of longer duration a steadily decreasing volume of the labelled P solution was injected at intervals of two hours all through the experiment. Due to the uptake of the labelled phosphorus by bone and other tissue, the ^{32}P concentration of the plasma strongly decreases during an experiment unless kept up in this way. In the case of sodium which is not taken up by the cells to any appreciable extent, the ^{24}Na concentration of the plasma does not much decrease with time.

In Table 1 it is seen that, while the apparent sodium space makes out 4 per cent only of the weight of the muscle after the lapse of 3 min, after 20 minutes 14 per cent are found, which almost corresponds to the actual sodium space. The fact that between 20 minutes and 4 days the ratio of the ^{24}Na content of plasma and muscle of equal weight hardly changes is showing clearly that no significant uptake of ^{24}Na by the muscle cells takes place in the course of 4 days.

TABLE 1. — DISTRIBUTION OF ^{24}Na AND ^{32}P, RESPECTIVELY, BETWEEN PLASMA AND GASTROCNEMIUS[1] OF EQUAL WEIGHT AT 22°

Time after administration of ^{24}Na and ^{32}P	$\frac{^{24}Na_m}{^{32}Na_p}$	$\frac{^{32}P_m}{^{32}P_p}$
3 min.	0.046	0.015
9 min.	0.12	—
20 min.	0.14	—
1 hour	—	0.155
10 hours	0.15	0.29
2 days	—	1.19
4 days	0.14	2.47

The subscripts m and p represent muscle and plasma, respectively

(1) The extracellular volume in per cent of the muscle weight is obtained by multiplying the above figures by 97 (see p. 445).

The amount of ^{32}P which penetrates into the muscle tissue in the course of the first 3 minutes is much smaller than that of ^{24}Na ; this phenomenon is due to a slower penetration of the phosphate ions through the capillary wall. After the lapse of 1 hour, a slightly greater percentage

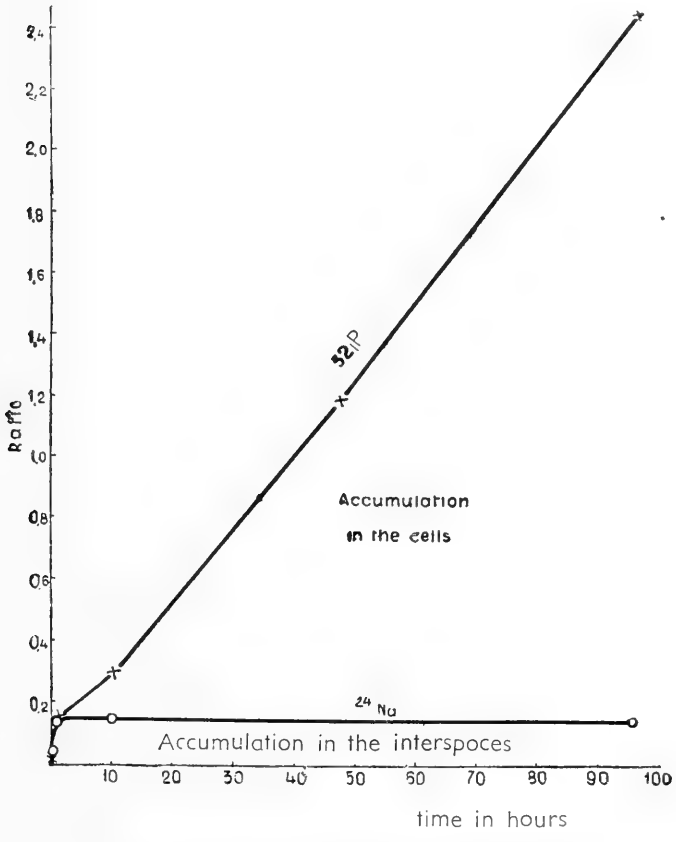

FIG. 1. Accumulation of ^{32}P in the cells of muscle tissue.

of the plasma ^{32}P is found in the muscle tissue than of the plasma ^{24}Na, the difference increasing with time. This phenomenon is due to a successive penetration of ^{32}P into the muscle cells. The amount migrated into the cells is obtained by subtracting the sodium space from the *apparent* phosphorus space. After 10 hours we find, as seen in Table 1 and Fig. 1, that the amount of ^{32}P which diffused into the cells of 1 gm gastrocnemius makes up 14 per cent of the ^{32}P content of 1 gm plasma or about 100 per cent of the ^{32}P present in the interspaces of 1 gm,

gastrocnemius. After the lapse of 4 days, the corresponding figures are 233 and 1653 per cent, respectively. The rate of penetration of phosphate ions into the muscle cells of the frog is, thus, a very slow one even at 22° and still slower at lower temperature. After the lapse of 10 hours at 0°, the *apparent* phosphorus space of the gastrocnemius was found to be 18 per cent ; thus, only about $^1/_5$ as much labelled phosphate diffused into the cells at 0° than 22°.

As mentioned above, we arrive at the values stated for the amount of labelled phosphate which migrated into the muscle cells by subtracting from the total amount of ^{32}P found in the muscle the amount of ^{32}P present in the interspaces. The accuracy of the figures obtained depends largely upon the accuracy of the figures assumed for the size of the interspaces.

In determining the extracellular volume we make two assumptions : a) We assume that all sodium or chlorine present in the muscle cells is exclusively found in the interspaces ; b) we assume the concentration of sodium and chlorine, respectively, to be the same in the plasma water and the extracellular fluid. Much evidence is available that these assumptions are essentially correct. It is possible, however, that a small amount of sodium or chlorine penetrates into the cells (Hastings and Eichelberger, 1937) and also that the extracellular fluid does not show exactly the same sodium or chlorine content as the plasma (comp. Manery *et al.* 1938). The size of the interspaces calculated from the distribution figures of sodium and chlorine, respectively, is, however, about the same. Fenn and Cobb (1936) found the average sodium space and the average chlorine space of the rats' muscle to be 12.6 and 11.4 per cent, respectively. Manery and Hastings (1939) state the apparent extracellular space of the gastrocnemius of the rabbit to be 11.3 per cent, calculated from the distribution of chlorine, and 11.0 per cent from the distribution of sodium, while for the abdominal muscle they give the figures 16.3 and 13.9 per cent, respectively.

We assume, furthermore, that an equal distribution of the labelled phosphate between plasma and extracellular fluid takes place at an early stage in the experiment. This assumption involves some uncertainty. After the lapse of 3 minutes (see Table 1), an equal distribution is far from being reached by either sodium or phosphate; after 1 hour equality may be reached also by the phosphate, but the possibility cannot be excluded that the equality of the sodium space and the phosphate space found after the lapse of 1 hour is a fortuitous one and is due to the fact that some ^{32}P penetrates into the cells before the equipartition mentioned above was obtained, the sum of the cellular and extracellular P present in 1 gm muscle making out 14 per cent of the ^{32}P content of 1 gm plasma. In the case of the brain tissue, the capillaries of which are only at a slow rate permeable to phosphate, we found obvious indications of a pene-

tration of ^{32}P into the cells before an equipartition of ^{32}P between plasma and extracellular fluid was obtained. It is, therefore, of importance to find a method which permits us to determine the amount of ^{32}P penetrating into the cells without having to make any assumption regarding the size of the interspaces and the time involved in obtaining an equal distribution of ^{32}P between plasma and extracellular fluid. Such a method will be described in the following section.

DESCRIPTION OF THE MODIFIED METHOD

When applying the modified method, we compare the activity of the inorganic P of a plasma sample of known weight with the activity of the *organic* phosphorus extracted from the muscle sample of the same weight. This method is based on the assumption that the organic phosphorus compounds present in the muscle tissue are formed in the muscle cells from inorganic phosphate and that, correspondingly, all active P atoms present in the organic constituents of the muscle are such which passed from the plasma into the cells as inorganic ^{32}P. Since some of the active phosphate penetrated into the cells will not have had opportunity to be incorporated into organic molecules, but will remain in inorganic state, the method here outlined will give a lower limit for the extent of phosphorus exchange between plasma and muscle cells. By adding to the activity of the organic P of the muscles that of the cellular inorganic P we arrive at the total cellular activity.

In experiments of several hours' duration or more, we can estimate the amount of cellular inorganic ^{32}P by the following consideration. Let us consider an experiment taking 10 hours. We find that, in this experiment, 66 per cent of the ^{32}P content of the acid soluble organic P of the muscle is present as creatinephosphoric acid P and that the amount of creatinephosphoric P makes out 2.4 times that of the inorganic P present as such in the muscle. The last mentioned data are obtained by the usual chemical determination of creatine P and inorganic P, respectively. Since from activity data we know that, in the course of 10 hours, almost all the creatinephosphate molecules present in the muscle get renewed, the ^{32}P content of 1 mgm inorganic P will be about equal te the ^{32}P content of 1 mgm creatine P. From these data it follows that the activity of the cellular inorganic P makes out $66 : 2.4 = 28$ per cent of that of the cellular acid soluble organic P. We have, thus, to add to the values obtained for the acid soluble organic cellular ^{32}P content of the muscle (see Table 3, column 2) 0.28 times the value obtained in order to get the value of the total ^{32}P migrated into the muscle cells during 10 hours. In an analogous way the other figures seen in column 2 of Table 3 were obtained. The correction

due to the presence of ^{32}P in the cellular inorganic P fraction was smaller in experiments of longer duration.

In the consideration stated above we have disregarded the fact that the amount of inorganic P extracted from the muscle is partly extracellular P. This procedure is permissible when dealing, as above, with chemical magnitudes alone in view of the fact that the amount of cellular inorganic P is about 60 times larger than that of the extracellular inorganic P of the muscle. We meet, however, very different conditions when considering the radioactivity of the cellular and extracellular inorganic P, respectively (which is not the case in the consideration made above). Due to the slow migration of the phosphate ion into the muscle cells, the activity of 1 mgm extracellular inorganic P may be many hundred times larger than that of 1 mgm cellular inorganic P.

Table 2. — Distribution of ^{32}P Between the Acid Soluble Organic Constituents Extracted from 1 gm Gastrocnemius and the Inorganic Phosphate Extracted from 1 gm Plasma

Time after administration of ^{32}P	Distribution coefficient	Percentage(1) of organic acid soluble P replaced by plasma P
10 hours	0.11	0.5
2 days	1.02	2.4
4 days	2.11	8.0

Table 2 contains data on the ratio of the activity of the inorganic P of plasma samples and that of the acid soluble organic P fractions isolated from muscle samples having the same weight as the plasma samples. These ratios are stated in column 2 and indicate, as mentioned above, the lower limit of the fraction of the plasma P which exchanged with cellular acid soluble P during the experiment. Column 3 contains data on the percentage of the organic acid soluble P which was replaced by plasma P during the experiment. In the course of 4 days, 8 per cent was replaced.

The amount of ^{32}P incorporated into the non-acid soluble P of the frogs' muscle is very restricted. After the lapse of 4 days, the number of ^{32}P atoms incorporated into phosphatides makes up 2.5 per cent of the amount present in the acid soluble compounds. For the ^{32}P incorporated into residual (protein) P, the corresponding figure was found

(1) The ratio of the acid soluble P content of 1 gm muscle and 1 gm plasma was found in the frog killed after 10 hours, 2 days and 4 days to be 31, 42 and 26.4 respectively. The variation of the above ratio is to some extent due to a variation in the acid soluble P content of the plasma which was found to be 3.6, 3.6 and 4.8 mgm per cent respectively.

to be 2.6 per cent. Thus, the amount of ^{32}P incorporated into all phosphorus fractions present in the muscle is, after the lapse of 4 days, but 5 per cent larger than the amount incorporated into the acid soluble compounds. In experiments of shorter duration the difference is still less.

TABLE 3. — DISTRIBUTION OF ^{32}P BETWEEN THE TOTAL CELLULAR ACID SOLUBLE PHOSPHATE EXTRACTED FROM 1 GM GASTROCNEMIUS AND THE INORGANIC PHOSPHATE EXTRACTED FROM 1 GM PLASMA

Time after administration	Distribution coefficient obtained by the modified method (organic fraction extracted, share of inorganic P computed)	Distribution coefficient obtained by the original method (direct comparison of the activity of muscle and plasma of equal weights after subtraction of ^{32}P content of the extracellular volume)
10 hours	0.14	0.14
2 days	1.20	1.05
4 days	2.50	2.33

In Table 3, the distribution coefficient of ^{32}P between cellular P and plasma P arrived at by the two different methods is given. Column 2 contains the distribution coefficient calculated from the ^{32}P content of the organic fractions and of the inorganic phosphate content of the muscle, as described on p. 449, while in column 3 the results are given which were obtained by comparing the activity of the muscle tissue with that of the plasma. The results obtained agree fairly well.

It is of interest to remark that the labelled phosphate was found to penetrate at a faster rate into the cells of mammalian muscle than in those of the frog (HAHN *et al.*, 1939). The ratio of the ^{32}P content of 1 gm gastrocnemius and of 1 gm plasma of the rabbit was found after the lapse of 4 hours to be 0.6.

DISCUSSION

The concentration of the inorganic phosphate present in the water of the muscle cells is about 100 times larger than that present in the plasma water. This puzzling difference can be explained in two different ways. We can assume that most of the inorganic phosphorus extracted after a most careful treatment of the muscle was not present as inorganic phosphate previous to extraction in the muscle but in the form of a very labile phosphorus compound. Creatinephosphoric acid is a fairly labile compound which can only be extracted without decomposition if the operation is carried out at a low temperature and a very fast

rate. It is quite conceivable that a decomposition of other still more labile phosphorus compounds during extraction cannot be avoided. But the existence of the great difference in the inorganic phosphate concentration of plasma and cell water can be explained in a very different way as well.

The muscle cells take up when formed a comparatively large amount of inorganic P. This high inorganic P content is maintained all through life, the cell walls being impermeable to phosphate ions or, as far as a restricted permeability is present, the phosphate lost by the cells is compensated by a secretion of an equal amount of phosphate from the plasma into the muscle cells. Numerous examples of such active secretion are reported by KROGH (1939), and the application of his views to the present problem leads to the last mentioned explanation.

It is not possible at present to decide which of the explanations mentioned above is the correct one ; the application of radioactive P as an indicator leads, however, to the result that a restricted permeability of the muscle cell wall to phosphate ions is actually present. Professor KROGH has kindly drawn our attention to a possibility of deciding which of these explanations is the right one. According to his view, the primary process is the loss of some cellular phosphate by leakage through the cell wall. The extent of the active secretion into the cells is that necessary to compensate for the loss by leakage and is determined by the extent of the latter. Let us increase the phosphate concentration of the plasma by administering large amounts of phosphate for example. This increase should, according to the view cited above, not influence the amount of labelled phosphate penetrating into the cells while, in the case that the entrance of labelled phosphate into the cells is due to diffusion, the amount entering the cells from a plasma containing more phosphate should be larger than from one containing less.

Summary

Labelled sodium and labelled phosphate are injected simultaneously into the lymph sack of the frog and the distribution of the radioactive sodium and the radioactive phosphate between plasma and muscle of equal weights is determined. A constant partition ratio of the radioactive sodium is obtained after the lapse of about 20 minutes. From this ratio the volume of the interspaces of the muscle can be calculated. In the case of phosphorus, the partition ratio increases even after the lapse of many days because of continued penetration of the labelled phosphate into the cells. The difference of the partition ratio of the radioactive phosphorus and the radioactive sodium permits us to calculate the amount of ^{32}P which penetrated into the muscle cells. Since all phosphorus atoms present in the plasma can be assumed to show the same behaviour as the ^{32}P atoms we can compute from the figures obtained the amount of plasma inorganic P which exchanged with cellular P during the experiment. In the course of 4 days at 22°

0.082 mgm P was found to penetrate into the cells of 1 gm gastrocnemius muscle and vice versa. At 0°, $^1/_5$ of the above value was found.

An alternative method wich is independent of the knowledge of the size of the extracellular space is based on the determination of the comparison of the active *inorganic* phosphorus content of the plasma with the active *organic* phosphorus content of the muscle. By this method, the amount of plasma P penetrated into 1 gm muscle in the course of 4 days was found to be 0.088 mgm.

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45. THE EFFECT OF EXCITATION ON NERVE PERMEABILITY

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The effect of muscular exercise on the permeability of muscular tissue to phosphates (Bollman and Flock, 1943 ; Hevesy and Rebbe, 1946) and potassium (Hahn and Hevesy, 1941 ; Noonan, Fenn and Haege, 1941) has previously been investigated by using ^{32}P and ^{42}K as indicators. Phosphate-permeability was found to be influenced only to a minor extent by muscular exercise, while the amount of potassium was found to be increased 3 to 4 times as the result on intense muscular work.

The present communication gives the results of experiments in which the effect of excitation on the permeability of the sciatic nerve for phosphate, sodium, potassium and bromide was investigated.

Radioactive isotopes were used as indicators.[1]

EXPERIMENTAL PROCEDURE

Cats weighing about 2 kgm were used. Chloralose, 5—6 ml of an 1 per cent solution per kgm was injected into a brachial vein under ether anaestesia, except in the first case (Table 1), where it was injected into the femoral vein on the stimulated side. The sciatic nerves were exposed near the spinal cord on each side and crushed with a forceps. Stimulation was effected by means of a thyratron stimulator giving condensor shocks at a rate of about 50 per sec. and at a strength producing about maximal motor reactions. In one experiment (sodium No 5) the animal was curarised before stimulation in order to avoid muscular movements.

The radioactive salts, dissolved in a few ml of distilled water, was injected intravenously through the cannula used for the anaesthetic. In the majority of cases the stimulation was carried on for 5 minutes and the active preparation injected at the end of the second minute of stimulation. Immediately after the stimulation a sample of heparinized blood was taken by heart puncture and centrifugated, the plasma being used for determination of the activity. The animal was killed by bleeding and the hind legs washed free of blood by perfusion with Ringer's solution through a cannula in the lower part of the aorta. When the

[1] Our thanks are due to Prof. Niels Bohr and to Prof. M. Siegbahn for the radio-active preparations kindly put at our disposal.

outflowing blood was macroscopically free from blood corpuscles the sciatic nerves on both sides were carefully dissected out and worked up.

Results

Phosphate Permeability

In Table 1 are given the weights of the fresh and dry nerve, the percentage of dry substance, the total phosphorus content of the nerve, the phosphorus content per gm fresh weight, the ratio of the activity per gm dry stimulated and non-stimulated sciatic nerve, the ratio of

TABLE 1. — WEIGHT OF CAT 2.4 KGM LEFT NERVE STIMULATED FOR 6 MIN. AT THE END OF THE FIRST MINUTE 0.75 MILLICURIE ^{32}P INJECTED INTRAVENOUSLY. INORGANIC PLASMA P = 6.10 MGM P. C.

	Fresh weight of sciatic nerve in mgm	Dry weight in mgm	Percentage dry substance	P content in μgm	mgm P per gm fresh nerve	Ratio of activities of 1 gm dry irritated and 1 gm non-irritated nerve	Ratio of activities of 1 μgm P extracted from irritated and non-irritated nerves	Percentage activity of 1 μgm nerve P to 1 μgm inorganic plasma P
Stimulated	415.7	135.4	32.6	2040	4.92			0.237
Non-stimulated ...	476.2	154.1	32.4	2110	4.04	1.18	1.10	0.216

TABLE 2. — WEIGHT OF CAT 2.95 KGM. LEFT NERVE STIMULATED FOR 5 MIN. AT THE END OF THE SECOND MINUTE 0.3 MILLICURIE ^{32}P INJECTED INTRAVENOUSLY. INORGANIC PLASMA P = 4.42 MGM P. C.

	Fresh weight of sciatic nerve in mgm	Dry weight in mgm	Percentage dry substance	P content in μgm	mgm P per gm fresh nerve	Ratio of activities of 1 gm dry stimulated and 1 gm non-stimulated nerve	Ratio of activities of 1 μgm P extracted from stimulated and non-stimulated nerves	Percentage activity of 1 μgm nerve P to 1 μgm inorganic plasma P
Stimulated	312.1	104.2	33.4	1320	4.18	2.46	1.98	0.080
Non-stimulated ...	394.6	127.4	32.0	1324	3.36			0.040

the activites of 1 μgm phosphorus extracted from the stimulated and non-stimulated nerve and finally the percentage activity of 1 μgm total nerve phosphorus relative to that of 1 μgm inorganic phosphorus.

It will be seen from figures in Tables 1 to 4 that the irritated nerve in each case took up more ^{32}P than the non-irritated nerve, the ratio

varying between. 1.18 and 2.46. For the ratio of the quotiens $\frac{^{32}P}{^{31}P}$ for the irritated and non-irritated nerves figures varying between 1.10 and 1.98 were obtained.

TABLE 3. — WEIGHT OF CAT 2.4 KGM LEFT NERVE STIMULATED FOR 5 MIN. AT THE END OF THE SECOND MINUTE 0.4 MILLICURIE ^{32}P INJECTED INTRAVENOUSLY

	Fresh weight in mgm	Dry weight in mgm	Percentage dry substance	P content in μgm	mg P per gm fresh nerve	Ratio of activities of 1 gm dry stimulated and 1 gm non-stimulated nerve	Ratio of activities of 1 μgm P extracted from stimulated and non-stimulated nerves	Percentage activity of 1 μgm nerve P to 1 μgm inorganic plasma P
Stimulated	—	117	—	1474			1.50	0.109
Non-stimulated ...	—	113	—	1350				0.075

TABLE 4. — WEIGHT OF CAT 1.8 KGM LEFT NERVE STIMULATED FOR 5 MIN. AT THE END OF THE SECOND MINUTE 0.4 MILLICURIE ^{32}P INJECTED INTRAVENOUSLY. DURING THE LAST MINUTE INTERMITTENT STIMULATION (2.5 SEC. IRRITATION AND 2.5 SEC. REST)

	Fresh weight of sciatic nerve in mgm	Dry weight in mgm	Percentage dry substance	P content in μgm	mg P per gm fresh nerve	Ratio of activities of 1 gm dry stimulated and 1 gm non-stimulated nerve	Ratio of activities μgm 1 μgm P extracted from stimulated and non-stimulated nerves	Percentage activity 1 μgm nerve P to 1 μgm inorganic plasma P
Stimulated	—	113	—	1048	—		1.35	0.137
Non-stimulated ...	—	101	—	1074	—			0.089

If we wish to know the percentage of P present in the nerve tissue (cf. above) that was taken up in the course of the experiment, we must compare the specific activity of the nerve P with the average value for the inorganic P of the plasma. This magnitude does not necessarily correspond to the amount that has penetrated from the plasma into the tissue during the experiment, as it is conceivable that a part of the P migrating from the plasma into the tissue cells has found its way back again. Considering that the amount of plasma P located in the nerve tissue constitutes only a small percentage of the total P content of the tissue and in view of the rapid participation of intruded phosphate in phosphorylation processes, we may suppose the amount of labelled P (plasma P) located in the nerve tissue to be practically identical with that penetrating from the plasma into the nerve tissue during the experiment. Assuming that the specific activity of the plasma inorganic P of the cat declines after intravenous injection at the same rate as in

a rabbit of the same weight, the average specific activity of the inorganic P in the plasma works out in a 3-minute experiment to be about 3 times that of the experimentally determined amount. For the amount of P penetrating during 3 minutes into the resting nerve tissue (cf. Tables 3 and 4) we hence obtain the values $\frac{0.08}{3}$ and $\frac{0.09}{3}$ as percentage activity of nerve P to plasma P. In the experiment recorded in Table 1 the approximate value $\frac{0.22}{5}$ is obtained. As discussed above the average specific activity of the plasma P is higher than its end value which was determined experimentally. To account for this decrease, we have in the experiment taken 5 minutes to divide the percentage activity of nerve P to plasma P with the figure 6 which should give an approximately correct value.

The amount of plasma P passing into 1 gm fresh resting nerve tissue in the course of 1 minute proves on the average to be 0.01 per cent of the total P of the nerve tissue, thus about 0.4 μgm.

The amount of phosphorus penetrating in 1 minute into the nerve tissue is smaller than the corresponding amount penetrating into the muscle cells. KALCKAR *et al.* (1944) estimate that, in the course of 1 minute, 1 μgm phosphorus penetrates into the cells of 1 gm fresh muscle tissue of the rat. A simular figure is reported by HEVESY and H. v. EULER (1942).

Sodium Permeability

In these experiments labelled sodium with an activity of about 0.5 millicurie was injected into the circulation. The cat was killed 5 minutes after the injection. In each experiment, as seen in Table 5, the irritated nerve was found to take up more ^{24}Na than the resting nerve.

TABLE 5. — UPTAKE OF ^{24}Na BY SCIATIC NERVE

	Weight of cat in kgm	Ratio of uptakes by irradiated and resting nerves	Activity of 1 mgm dry nerve as percentage of activity of 1 mgm dry plasma	
			irritated	resting
I.	3.5	1.40	0.77	0.55
II.	1.85	1.56	1.38	0.81
III.	1.9	1.06	2.67	2.48
IV.	2.2	1.98	4.80	2.42
V.	1.85	1.32	1.79	1.36

Assuming 1 mgm. dry plasma to contain 40 μgm. sodium and the mean activity of sodium during the 5-minute experiment to amount to 3

times that of the activity measured at the end of the experiment, 1 mgm dry resting nerve takes up on an average 0.2 μgm sodium per minute. In view of the very rapid change in the ^{24}Na content of plasma which follows intravenous injection, this figure represents only a rough estimate of the amount of sodium taken up by the nerve.

Potassium Permeability

In these experiments labelled potassium chloride having a specific activity of 0.3 millicurie was injected. The injection took 1 minute, the cat was killed 2 minutes later. The results obtained are seen in Table 6.

TABLE 6. — UPTAKE OF ^{42}K BY SCIATIC NERVE

Weight of cat in kgm	Ratio of uptakes by irritated and resting nerves	Activity of 1 mgm dry nerve as percentage of activity of 1 mgm dry plasma	
		irritated	resting
I. 3.0	2.29	6.62	2.88
II. 1.9	1.51	8.60	5.83

By a similar consideration as put forward in the case of sodium we arrive to the conclusion that in the course of 1 min. about 0.02 μgm potassium penetrates in 1 mgm dry nerve tissue or 6 μgm in 1 gm fresh tissue.

It is of interest to compare this figure with the data recently obtained by HODGKIN and HUXLEY (1946) when determining the number of moles of potassium which leak through 1 cm^2 of membrane of the axons from *Carcinus maenas* in one impulse ($1.7 \cdot 10^{-12}$) and the amount of potassium re-absorbed during the period of recovery. When the external potassium concentration is increased threefold, t3.10 $^{10}\gamma$ mol cm^{-2} sec^{-1} or 0.7 μgm potassium per minute were found to be re-absorbed.

Bromine Permeability

In these experiments 30 mgm bromine as sodium bromide, with an activity of 0.2 millicurie was injected.

TABLE 7. — UPTAKE OF ^{82}Br BY SCIATIC NERVE

Weight of cat in kgm	Ratio of uptakes by irritated and resting nerves	Activity of 1 mgm dry nerve as percentage of activity of 1 mgm dry plasma	
		irritated	resting
I. 1.98	1.34	3.4	2.9
II. 2.2	1.72	4.0	2.3

Assuming, as in the case of ^{24}Na, that the average plasma activity during the experiment amounts to about $^1/_3$ of the determined end activity, 1 mgm. dry resting nerve took up 0.01 μgm. bromine. Similar amounts of radiobromine were found to be taken up by the basal ganglia, cerebreal cortex and the medulla oblongata of the cat.

In no single case did the stimulated nerve fail to show an enhanced uptake of the ion investigated. When we compute the mean of all determinations (13 cases) of the ratio of uptake of various ions by the stimulated and the resting nerves, the value 1.55 ± 0.10 is obtained. The uptake by the stimulated nerve was also found to be enhanced when the cat was curarized before stimulation in order to avoid muscular movement.

Summary

The effect of stimulation on the amounts of phosphate, sodium, potassium and bromide taken up by the sciatic nerve of the cat was investigated with the aid of radioactive isotopes as indicators. Stimulation was effected by condensor shocks at a rate of 50 per sec. giving maximal motor reactions.

In each case investigated, including the curarized animal, the stimulated nerve was found to take up more labelled ions than the resting nerve, the mean ratio of the uptakes being 1.55 ± 0.10.

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46. NOTE ON THE INORGANIC PHOSPHATE OF BLOOD PLASMA

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In tracer work with radiophosphorus, we often add labelled sodium phosphate to blood plasma or inject it into the circulation and follow by means of radioactive measurements the path of the tagged phosphate into the red corpuscles, their passage through the capillary wall or other phase boundaries. For this type of work it is of great importance to know whether the inorganic phosphate, added or injected, shows the same behaviour as the endogenous inorganic phosphate present in the plasma. Should that not be the case, the calculation of the amount of phosphate penetrating from the plasma into the red corpuscles, for example, from the distribution date of the ^{32}P added and the inorganic phosphate of the plasma would clearly lead to wrong results. To investigate if and to what extent added and endogenous inorganic phosphate show a different behaviour, we added to heparinized human or cat plasma a tracer dose of sodium phosphate of negligible weight and, after rotating the plasma at 37° for 1 hour in a thermostat, we electrodialysed the plasma. At intervals varying between 1 and 18 hours, we took samples and determined by radioactive measurements and colorimetric determinations, respectively, at what rate the added labelled phosphate and the endogenous inorganic P, respectively, is removed from the plasma.

EXPERIMENTAL

The dialysator applied was of the Hahn—Tiselius (1943) type. As membrane a thin sheet of cellophane was used. To 20 ml heparinized plasma an equal volume of physiological sodium chloride solution containing about 10 γ labelled phosphate of ½ microcurie activity was added. To keep the salt content of the plasma at constant level a diluted sodium chloride solution was added at intervals. Through the electrode cells a borate buffer solution of pH 7.6 circulated. The potential applied to the ice-cooled dialysator was 20 Volts; the current intensity amounted to 30 milliamp. The inorganic phosphate was extracted from the plasma with 10% trichloroacetic acid. We carried out extractions both at 0° and at 20°. Imperfect extraction would not influence our results, as aliquots of the same extract were applied to the radioactive measurements and to the colorimetric determinations.

In some cases, the amount of the total acid-soluble P present and its activity were determined as well. In these experiments, the acid-soluble extract was ashed and an aliquot used to colorimetric determination of the total (inorganic + organic) acid-soluble P present, another aliquot to radioactive measurements.

RESULTS

Numerous experiments were carried out leading to the result that a difference is present between the behaviour of the inorganic phosphate added and the endogenous inorganic phosphate, but the difference is

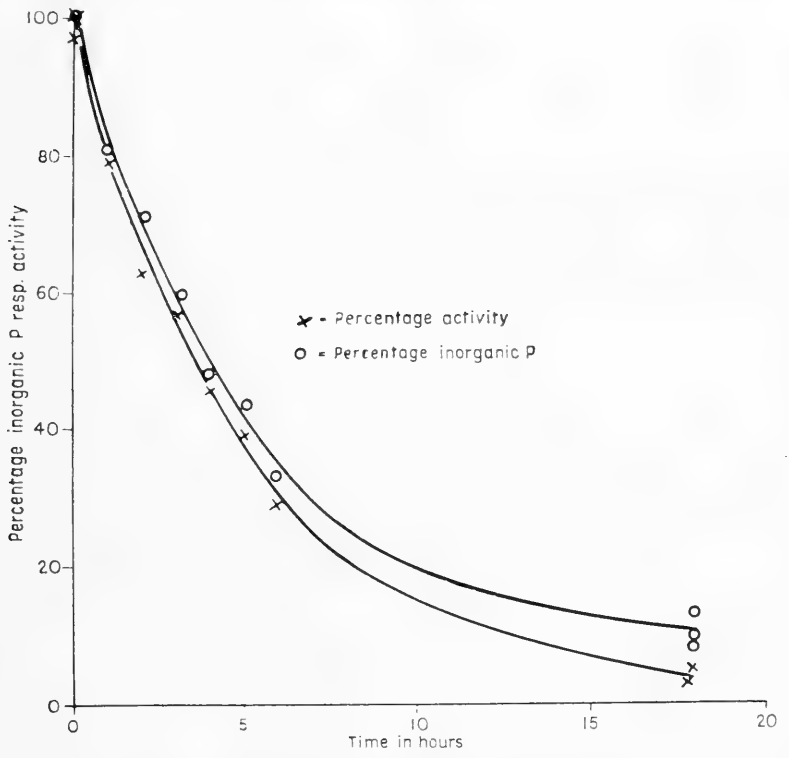

Fig. 1. Rate of disappearance of the endogenous inorganic P content of human plasma and of the radiophosphorus added.

not pronounced. As seen in Fig. 1 and other similar figures obtained by us, the radioactivity of the plasma sample declines at a somewhat more rapid rate than its inorganic P content. After the lapse of 18 hours 10% of the inorganic P is still present, but only 4% of the activity added. Thus, about 6% of the endogenous plasma inorganic P do not dialyse and, consequently, do not get into exchange equilibrium with the labelled phosphate of the plasma. In some experiments, up to 10% of the endo-

genous inorganic P were found not to dialyse. The non-dialysing inorganic phosphate is presumably combined with proteins. That a minor part of the inorganic P of the plasma is bound to proteins is also made very probable by previous work in which other methods were used and which are discussed below.

As seen in Table 1, the total acid-soluble P content was found to be appreciably higher than inorganic P content, about $^1/_5$ of the acid-soluble P being present in organic binding. The specific activity of the total acid-soluble P was found to be lower than the corresponding value of the inorganic P, indicating that the labelled phosphate does not interchange or interchange to a minor extent only with the organic P present in the plasma.

PREVIOUS WORK ON THE PRESENCE OF PHOSPHATE-PROTEIN COMPOUNDS IN THE PLASMA

Macheboeuf and Sørensen (1925/27) in their studies on the composition of egg-albumin were led to the result that between the large complexes which are constituting egg-albumin molecules few phosphorus containing complexes are present; these are bound so firmly to the other groups of the molecule that they have to be considered a constituent of the latter. The amount of protein-bound phosphorus was found in albumin, resp. globulin to amount to 7.5 resp. 2 mgm per gm total nitrogen. This phosphorus could not be separated from albumin, resp. globulin, by electrodialysis. Sørensen (1925/27) investigated on similar lines the phosphorus content of serum-albumin and serum-euglobulin. Serum albumin and euglobulin were found to contain 2—40, resp. 0.15–0.3 mgm P per gm total nitrogen. In contrast to the phosphorus of egg proteins only a small percentage of the phosphorus of the serum proteins could be precipitated by alcohol. This and other observations induced Sørensen to regard the serum protein P as an accessory constituent of the serum proteins only. No conclusions can be drawn from these investigations if this accessory phosphorus is getting into exchange equilibrium with the comparatively large amounts of inorganic phosphorus simultaneously present in the plasma or not.

Masket *et al.* (1942) analysed fractioned centrifuged horse serum and found that the inorganic phosphorus concentration increases progressively with the protein concentration. Differences of 0.14 and 0.15 mM of P per kilo water were obtained between the top and bottom fraction, the top fraction containing 11% less, the bottom fraction 12% more inorganic P than the unfractionated plasma which contained 1.3 mM per kilo of H_2O. These authors conclude from their results that phosphate-protein compounds normally occur in horse serum.

That protein bound inorganic phosphate is present in small amount only in the serum of the dog is also borne out by the work of SMITH *et al.* (1943) who determined the percentage of ultrafilterable inorganic serum phosphate in 13 cases. The average percentage of ultrafilterable inorganic P found in their experiments works out to be 96%.

TABLE 1. — INORGANIC P CONTENT AND ACTIVITY OF PLASMA SAMPLES SECURED FROM THE DIALYSATOR AT VARIOUS TIMES

Time in hours	Inorganic P content in relative units (100 relative units = 3.7 mgm%)	Activity in relative units (100 relative units = 200 counts per min
0	100	100
2	69.3	63.2
3	58.5	56.7
4	47.1	45.6
5	42.6	39.2
6	32.7	29.3
18[1]	9.8	3.2
18[1]	9.1	3.9
18[2]	12.7	4.4
	Total acid-soluble P	Specific activity of total acid-soluble P / Specific activity of inorganic P
0	127	0.80

[1] Extracted at 20°.
[2] Extracted at 0°.

Not only a combination with proteins, but also the formation of colloidal phosphate will prevent ultrafiltration of inorganic P or its removal into the electrodialysate. GROLLMAN (1927) found in an early work that, while the inorganic P of normal pig serum is entirely ultrafilterable, a successive increase in the calcium content of the plasma from 9.4 to 32.2 mgm % makes the inorganic phosphate less and less ultrafilterable and, finally, only 5% are found in the ultrafiltrate. Ample evidence was brought by different authors that only excessive quantities of calcium phosphate salts lead to the formation of detectable amounts of a colloid complex (cf. SCHMIDT and GREENBERG, 1935; MCLEAN and HINDRICHS, 1938).

GOVAERTS (1943, 1947) compared the specific activities of the inorganic P of plasma and urine shortly after intravenous injection of labelled phosphate into the dog. In the first 1½ hours, the specific activity of the urine P was found to be greater than the corresponding value of the plasma P; after the lapse of that time no difference was found. GOVAERTS interprets these results as indicating that the greater

part of the acid-soluble P of the plasma does not get into exchange equilibrium with the injected inorganic phosphorus.

Our results do not contradict those of GOVAERTS. Phosphate identified after treatment of the plasma with trichloracetic acid as inorganic phosphate but actually present in the plasma in a labile low molecular organic binding may show a similar electrodialytic behaviour as does inorganic phosphate. Proofs of the presence of a labile phosphorus compound in the plasma is yet outstanding.

Summary

To determine whether inorganic labelled phosphate added to plasma gets into exchange equilibrium with inorganic phosphate present previously, labelled phosphate of negligible weight was added to human plasma. The plasma was electrodialysed, samples were taken from the dialysator at intervals, their inorganic phosphorus content and its radioactivity determined.

No pronounced difference was found in the rate of disappearance of the inorganic P content and of the radioactivity of the sample. As, however, after the lapse of 18 hours, only 4% of the original activity, but 10% of the original inorganic P content were present, we have to conclude that a small percentage of the plasma inorganic phosphate, possibly combined with proteins, does not interchange with the labelled phosphate added.

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47. FATE OF THE SULPHATE RADICAL IN THE ANIMAL BODY

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Phosphorus enters as phosphate in the numerous compounds in which it is to be found in the animal body; in connexion with the investigations carried out in recent years concerning the fate of ingested phosphorus atoms in the organism, it seemed to be of interest to determine whether or not, in the course of the numerous metabolic processes in which phosphorus is involved, the phosphate radical exchanges its *oxygen* content with other oxygen atoms present in the body. This question could be answered by injecting into an animal sodium phosphate which contained heavy oxygen (^{18}O) as an indicator and then determining if the phosphate recovered in the urine, for example, contained more than the normal amount of ^{18}O.

As, however, it was recently found[1] that "heavy-oxygen phosphate" can be obtained by dissolving sodium phosphate in "heavy-oxygen water" and vice versa, it is apparent that the oxygen atoms present in phosphate radicals exchange their places freely in water and there can be scarcely any doubt that the probability is extremely small of a phosphate radical leaving the body coupled to the same oxygen atoms with which it entered. Sulphate ions, on the other hand, have been found[2] to exchange oxygen atoms either not at all or at a very slow rate with neutral water, even at 100° C., and it seemed of interest, therefore, to investigate whether sulphate ions during their circulation in the body participate in chemical reactions which loosen the oxygen bonds sufficiently to make an oxygen exchange possible.

In the experiments we wish to report here, sodium sulphate containing heavy oxygen was prepared from heavy-oxygen water, kindly presented to us by Prof. Urey[3] having a density 740 parts in a million greater than that of normal water. The reaction used for the preparation of the "heavy sulphate" was that which takes place between SO_2Cl_2 and heavy-oxygen water in the presence of traces of iodine as a catalyst. 1 gm. of the dry material, converted into 50 cc. of solution, was injected into a rabbit. The urine of the rabbit was then collected for 24 hours, its sulphate content recovered as barium sulphate, the oxygen content

of the latter converted into water, and the density of this determined. The preparation of water from the oxygen of the sulphate was carried out in the following way. The barium sulphate precipitate was dried at 400° C. in a stream of nitrogen and then reduced with purified carbon at 900° C.; the gases evolved were mixed with a great excess of hydrogen and stored over oil in a gasometer; and, finally, the gas mixture was led over a nickel catalyst at 310° C. and the water formed collected. The density determination was kindly carried out by Mr. O. Jacobsen, using Linderström-Lang's floating-drop method.

Should the sulphate oxygen, during its stay in the animal, enter into exchange reactions with other oxygen atoms present in very great excess in the body, the oxygen of the heavy radicals would be replaced by normal oxygen atoms and the water prepared from the sulphate recovered from the urine would show the density of normal water. If, on the other hand, the sulphate ions injected retain the oxygen atom with which they start, the water prepared from the urine sulphate should show an excess density of 370 parts per million if no secretion of normal sulphate took place. The water prepared from the sulphate isolated from the urine after injecting heavy-oxygen sulphate has shown a very appreciable density excess — 240 parts per million. When comparing this value with the one calculated on the assumption that no exchange of sulphate oxygen took place, we must consider the following fact. Besides the heavy-oxygen sulphate — 0.84 gm of sodium sulphate being secreted in all during the day following injection — the urine contains also sulphate, even when no injection is given, the amount of which we found to correspond to 0.23 gm per day. The latter is normal sulphate and its presence reduces the density excess of the water prepared from the urine sulphate.

From the high density found for the water prepared from urine sulphate, one must conclude that most of the individual sulphate ions injected into the rabbit are recovered in their original form, and from this it follows that at least the greatest part of the sulphate administered leaves the body unchanged.

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4. Manian, Urey and Bleakney, *J. Amer. Chem. Soc.* **56,** 2601 (1934)

Comment on papers 42—47

When first investigating the rate of exodus of ^{24}Na from the plasma of the rabbit, we were struck by the rapidity with which this takes place, by the swiftness of the interchange between vascular and extravascular sodium. After the lapse of ½ min. about half of the former was replaced by the latter. We know today that the figures obtained in such investigations indicate only the lower limit of the speed with which such an interchange takes place. The injected labelled sodium has first to diffuse into the capillaries before leaving the circulation, and the interchange between vascular and extravascular sodium which takes place during this early interval is not indicated by the tracer.

When carrying out our first experiments we had at our disposal potassium of very low specific activity only. To carry out an experiment on the rate of exodus of ^{42}K from the circulation, we had to inject very rapidly 10 ml of physiological potassium chloride solution. Though the blocking effect of potassium on heart beat is well known, we were much impressed by the momentary fatal effect of such a rapid injection of a potassium chloride solution. When in possession of potassium of higher specific activity, we compared the rates at which sodium and potassium ions leave the circulation. That the latter was found to take place at a more rapid rate is presumably mainly due to the following fact: The labelled ions leave and re-enter the circulation. The re-entry is facilitated in the case of sodium by the restricted extravascular pool, sodium being mainly an extracellular element in contrast with potassium. Potassium ions which left the plasma and entered tissue cells have less chance of re-entering the plasma than have sodium ions.

As shown in paper 43 in ½ min half of the labelled water leaves the plasma of the rabbit. This is, for the reason mentioned above, an upper limit of the half-time that the water molecules remain in the plasma. After the lapse of 1 hr an almost complete equipartition of the water molecules between the circulation and the extravascular water was observed.

Since radio-sulphur was not available at that date for the study of the fate of the sulphate group in the organism, we applied with ^{18}O labelled sulphate (paper 47).

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48. DIPLOGEN AND FISH

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In recent months we have been carrying out experiments on the behaviour of fish in heavy water. We find that goldfish *(Carassius auratus)* behaved quite normally in the heavy water in which they were kept. As heavy water was to be used as indicator of normal water, we had to carry out our experiments in water containing only 0.5 mol. per cent of diplogen, and it is therefore still possible that a higher concentration of this isotope in water exerts effects upon fish.

The aim of our experiments was to follow the exchange of water between the fish and their surroundings, using heavy water as an indicator of the movement of the total water. The use of radioactive isotopes for such purposes is well known. While the latter are practically chemically identical, and as such are entirely trustworthy indicators, that is not the case with the isotopes of hydrogen. Heavy water is, therefore, only to be used with great caution as an indicator of ordinary water. However, when using very dilute solutions of heavy water, we may expect that the rate of exchange of heavy water molecules between the fish and its surroundings will not be very different from that of the normal water molecules. By measuring the speed at which the heavy water enters the body of the fish we can therefore conclude at what rate approximately the exchange of water between the fish and its surroundings takes place.

Some twenty fish having a total volume of about 10 cc. were kept in about 60 cc. of water containing 0.5 mol. per cent diplogen water. After

Table 1. — Rate of Entrance of Heavy Water into Fish

	Time in hours	Decrease of the heavy water content of the surrounding water	Decrease expected in the case of equal distribution of the heavy water between fish and surrounding water
I	1	32 p.c.	30 p.c.
II	4	32 p.c.	29 p.c.
III	15	33 p.c.	30 p.c.

a certain time the fish were removed and the decrease of the density of the surrounding water was determined. The fish were then placed in normal water, and the rise in the density of the latter due to the entrance of heavy water molecules leaving the body of the fisch was determined. The results are shown in the accompanying tables.

TABLE 2. — RATES OF LOSS OF HEAVY WATER BY THE FISH

	Time in hours	Initial heavy water content of the fish	Decrease of the heavy water content of the fish after the experiment	Decrease expected in the case of equal distribution of heavy water between fish and surrounding normal water
I	1	0.27 p.c.	68 p.c.	51 p.c.
II	4	0.27 p.c.	68 p.c.	67 p.c.
III	10	0.26 p.c.	86 p.c.	86 p.c.

It follows from the above that, at least in a small fish, within a few hours all the water molecules leave the body of the fish, making way for water molecules derived from the surrounding water. It should be borne in mind that most fish contain about 80 per cent water.

Comment to paper 48

Urey's discovery of heavy water was bound to impress the tracer-minded scientists, although their number was very restricted in those days. The present writer at once approached Professor Urey who most generously mailed a few litres of water containing 0.5 mol. per cent heavy water. In view of the great sensitivity with which the density of water can be determined, this strongly diluted heavy water sufficed to study the interchange between the water molecules of the goldfish and the surrounding water, and also to carry out studies described in paper 48, and presented more in detail by Hevesy and Hofer (1934). Within 1 hr an almost quantitative interchange between the water molecules of the fish and those of the surrounding water was found to take place. The amount of deuterium incorporated into the organic components of the fish, compared with the amount of deuterium entering as deuteriated water into the fish, was found to be small. The present writer intended, if successful in obtaining more concentrated heavy water, to study this type of incorporation. Shortly afterwards, Schoenheimer and Rittenberg embarked on the study of this problem and solved it in a masterly way (cf. p. 403). The discovery of artificial radioactivity induced the present writer to abandon his original plans and to find out if and to what extent the mineral constituents of the skeleton are in a dynamic state cf. Radioactive Indicators in the Study of Phosphorus Melambolism in Rats.

In paper 48 it is stated that the goldfish behaves in the same way in the heavy water employed in the experiments described as in tap water, though it may behave differently in more concentrated heavy water. In experiments with Häggkvist carried out in recent years (1958), we found that the life-span of the fish investigated was reduced from years to 10 days when kept in 40 per cent heavy water. When the fish were placed in 50 per cent heavy water, they tried to escape by jumping out from the vessel in which they were kept.

When our paper on the interchange of the water molecules of the goldfish and those of the surrounding water was published, Urey had not yet proposed a name for heavy hydrogen, while Rutherford discussed the possibility of calling it diplogen. This explains why we chose "Diplogen and fish" as the title of our paper.

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49. INTERACTION OF PLASMA PHOSPHATE WITH THE PHOSPHORUS COMPOUNDS PRESENT IN THE CORPUSCLES

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LIST OF SYMBOLS

p — total amount of plasma-phosphate;
c — total amount of acid-soluble phosphorus in corpuscles
s_p — specific activity of plasma phosphate (activity per mgm phosphorus);
s_i — specific activity of inorganic phosphorus in corpuscles;
s_c — average specific activity of total acid-soluble phosphorus in corpuscles;
a_p — total activity in plasma phosphate;
a_c — total activity of acid-soluble phosphorus in corpuscles;
S_p — value of s_p at the end of an experiment;
S_i — value of s_i at the end of an experiment;
S_e — specific activity of acid-soluble organic phosphorus in corpuscles at the end of the experiment;
S_c — value of s_c at the end of an experiment, etc.;
A_t — total activity of plasma ester at the time;
A_o — total activity of plasma ester at the start of the experiment;
α — coefficient of penetration;
k — rate-constant of the monomolecular decomposition of hexosephosphate in blood.

The distribution of inorganic phosphate and of inorganic acid-soluble phosphorus compounds between plasma and corpuscles deviates from equipartition. This difference in the distribution of the phosphate ion could be due to the fact that during the life time of the corpuscles equilibrium between its contents and those of the surrounding plasma is not yet reached, but it is more probable that we are faced with a case in which the partition coefficient of the ion in question between corpuscles and plasma actually differs from unity.

The distribution coefficient of inorganic phosphate and also that of the acid-soluble organic phosphorus compounds between plasma and

corpuscles fluctuates within wide limits. HALPERN[1] investigated the inorganic P content of the plasma and corpuscles of rabbit blood in 33 cases. In 29 cases the inorganic P content of the corpuscles was found to be less than that of the plasma of equal volume, the ratio between the inorganic P content of the corpuscles and that of the same volume of plasma varying between 0.86 and 0.38. In our experiments we find an average content of the plasma inorganic P amounting to 7 mgm % and of the corpuscles inorganic P to 4.5 mgm % making the above mentioned ratio equal to 0.64. When taking into account that the water content of the corpuscles amount to only 70% of that of the plasma, we obtain for the distribution coefficient of the inorganic P between the corpuscle water and plasma water a value differing not much from unity.[2] While the determination of the inorganic P of the plasma is not difficult, as the acid-soluble plasma P is mostly composed of phosphate ions, the analysis of the corpuscles often gives less reliable results. Some of the organic phosphorus compounds present in the corpuscles may decompose[3] in the course of the formation of additional inorganic phosphate. On the other hand, when the corpuscles have to be obtained quite free of plasma as in our experiments, it is necessary to wash them with a suitable solution free of phosphate, for example with an isotonic sodium chloride solution in the course of this operation some phosphate can be lost from the corpuscles by a diffusion process. In view of the great importance the plasma phosphoric esters play in ROBISON's theory of bone calcification, he and his collaborators[4] made a careful study of the amount of phosphoric ester present in the plasma from human and rabbit bloods and ascertained an average value of about 0.5 mgm%.

The problem in which we were interested was the determination of the rate at which phosphate ions and also the phosphoric ester molecules of the plasma penetrate into the corpuscles and vice versa. The usual procedure employed to obtain information on the permeability of the

[1] L. HALPERN, *J. Biol. Chem.* **114,** 747 (1936). In this paper earlier literature on this subject is to be found. R. T. BRAIN, H. O. KAY and P. G. MARSHALL [*Biochem. J.* **22,** 628 (1927)] found the inorganic P content of the human blood plasma to be 4.1 mgm. %, that of the corpuscles 2.4 mgm. %.

[2] R. T. BRAIN, H. O. KAY and P. G. MARSCHALL [*Biochem. J.* **22,** 629 (1928)] find for human blood the same distribution coefficient as found by us for canine blood, namely 0.91.

[3] MAITLAND, HANSMAN and ROBISON [*Biochem. J.* **18,** 1152 (1924)] have shown that, of the blood is made acid to pH — 7.3, there is hydrolysis, if made alkaline to pH — 7.35 there is for a short time synthesis of the esters; this, however, soon gives place to hydrolysis and to a corresponding increase of the inorganic phosphate; comp. also H. LAWACZECK, *Biochem. Zeitsch.* **145,** 351, 1924.

[4] R. ROBISON, *The Significance of Phosphoric Esters in Metabolism*, p. 68., New York (1932) Comp. also R. T. BRAIN, H. O. KAY and P. G. MARSHALL, *loc. cit.*

corpuscle membrane to phosphate ions is to introduce sodium phosphate into the plasma and to investigate if and to what extent the phosphate and phosphoric ester content of the corpuscles is increased. By using this line of attack HALPERN found that at 3° inorganic P does not enter or leave the blood cell to any appreciable extent in the course of 9 hours. Above 23° a very slow, at 37° an appreciable penetration of the additional phosphate into the corpuscles was observed.

A very convenient way of the study of exchange between phosphorus components present in the plasma and the corpuscles is opened by the application of labelled (radioactive) phosphate. By introducing active sodium phosphate of negligible weight into the plasma, all the phosphate ions present in the latter get labelled and, if after the lapse of some time radioactive phosphorus compounds are found to be present in the corpuscles, we can conclude that these penetrated during the time in question from the plasma into the corpuscles. We carried out experiments both *in vivo* and *in vitro*, introducing active sodium phosphate into the plasma and investigating, after the lapse of a few hours, the activity and the concentration of the inorganic phosphate and also of the phosphoric esters present in plasma and corpuscles. In other cases active hexosemonophosphate was introduced into the plasma and the activity and concentration of the above mentioned P compounds were measured. In view of the very slow rate of the formation of labelled "non-acid soluble" phosphorus-compounds present in the blood (phosphatides and phosphorus containing proteins) ascertained in our former work[1] we left those substances out of consideration in this investigation. Some of the acid-soluble phosphorus compounds, of which a great variety occurs in the corpuscles, were found to be labelled to a large extent after the lapse of a short time. The problem which first occurs is whether the labelled organic phosphorus compounds (phosphorus esters and others which we will in what follows denote as "phosphorus ester") are formed within the corpuscles from active inorganic phosphate or whether active esters diffuse from the plasma into the corpuscles. As we will see later, the labelled esters found in the corpuscles are, at least to a large extent synthesized within the corpuscles.

As to the nature of phosphoric esters present in the corpuscles, the presence of various compounds has been recorded, such as adenosintriphosphate, hexosephosphate, triosephosphate, mono- and diphosphoglycerate, glycerophosphate, and phosphopyruvate. The composition of the corpuscles of different animals was found to be markedly different ; while the corpuscles the blood of sheep contain,[2] for example, 80% of esters which are hydrolysed by boiling 1 n. HCl within 3 hours, the

[1] L. HAHN and G. HEVESY, *Mem. Carlsberg Lab.* **22,** 188 (1938).
[2] H. v. EULER and K. M. BRANDT, *Z. physiol. Chem.* **240,** 215 (1936).

corresponding figure amounts, in the case of rats' blood, to only 30. The average ester P content of human blood corpuscles is stated to be 20 mgm per 100 cc. blood, of which about 68% is present as phosphoglycerate, 21% as hexosephosphate, and 11% as adenyltriphosphate.[1] Few data are available as to the phosphorus ester content of the plasma and its composition, the presence of small amounts of hexosemonophosphate being recorded[2]. The phosphoric ester content of the plasma varies within wide limits, the average value being about 0.5 mgm% In the normal human plasma values varying between 0.0 and 0.9 mgm% and an average valve of 0.33 mgm% were recorded[3].

DIFFUSION OF PHOSPHATE IONS INTO THE CORPUSCLES

Radioactive sodium phosphate containing a negligible amount of phosphorus is added to 10 cc. of heparinised rabbit blood. The sample is shaken in a thermostat at 37° under a mixture of oxygen and carbon dioxide, after the lapse of few hours plasma and corpuscles are separated by centrifuging, the corpuscles washed 2—3 times with a physiological sodium chloride solution. The acid soluble components of the plasma and also those of the corpuscles are isolated in the usual way (extraction with ice-cold trichloro-acetic acid). While the acid-soluble fraction of the plasma is practically (90% or more) composed of inorganic P, the corpuscles contain mostly organic phosphorus compounds and, in addition, some inorganic P. The latter can be isolated by precipitation as ammonium magnesium phosphate. The organic phosphorus compounds present in the filtrate are then converted into inorganic salts and precipitated as such.

When carrying out such experiments, we find that in the course of few hours an appreciable part of the labelled plasma inorganic phosphate penetrates into the corpuscles. At the same time we find a formation of labelled organic phosphorus compounds in the corpuscles. What actually happens is that the individual inorganic phosphate ions of the plasma diffuse into the corpuscles and are converted in the latter into phosphorus esters. The question, which now occurs, is which is the faster process, the diffusion of HPO_4'' into the corpuscles or the ester formation. This can be decided by comparing the specific activities of the different phosphorus fractions isolated from the corpuscles. Such a comparison is seen in Table 1. After the lapse of only half an hour about half the

[1] E. Warweg and G. Stearns, *J. Biol. Chem.* **115,** 567 (1936).
S. E. Kerr and A. Antaki, *J. Biol. Chem.* **121,** 531 (1927).
[2] Comp. R. Robison, *The Significance of Phosphoric Esters in Metabolism* p. 69. New York (1932).
[3] R. T. Brain, H. D. Kay and P. G. Marshall, *Biochem J.* **22,** 635 (1928).

ester was in exchange equilibrium with the inorganic P present in the corpuscles. The re-synthesis of the acid soluble organic P compounds must thus be a fast process and, from the fact that with increasing time the ratio between the specific activity of the ester P and inorganic P of the corpuscles only slightly increases and yet strongly differs from 1,

TABLE 1. — ACTIVATION IN VITRO OF ACID-SOLUBLE PHOSPHORUS PRESENT IN THE CORPUSCLES

Relative specific activities of corpuscles' phosphate and of organic acid-soluble phosphorus present in the corpuscles, taking the specific activity of the plasma-phosphate at the end of the experiment to be = 1.

	Time	Rel. spec. activity of corp. phosphate $\left(\frac{S_i}{S_p}\right)$	Rel. spec. activity of corp. org. acid-soluble P $\left(\frac{S_e}{S_p}\right)$
Rabbit G	90 min.	0.25	0.14
	90 min.	0.38	0.21
Rabbit H	30 min.	0.27	0.11
	90 min.	0.46	0.23
	175 min.	0.69	0.36

we must conclude that only a part of the diverse organic phosphorus compounds present in the corpuscles is renewed and thus activated in the course of the experiment while the other part, composed of diphosphoglycerate, hexosephosphate and other compounds remains, at least practically, inactive. This conclusion is supported by the results of the following experiments. Instead of destroying the total esters we hydrolysed[1] them with 1 n. HCl or H_2SO_4 for 100 min at 100° and determined the specific activity of the hydrolysed P. While the average ester P secured from the corpuscles of a rabbit (G, comp Fig. 1) was found to have a specific activity amounting to 55% of the corpuscle inorganic P the corresponding figure for the hydrolysable ester was 80%. In the case of another rabbit (H), the figures were 53% and 100% respectively[2]. From the facts mentioned above, it follows that the exchange

[1] Under these conditions, diphospho-1-glycerate and also hexosediphosphate are only hydrolysed to a negligible resp. small extent (G. WARWEG and E. STEARNS, *J. Biol. Chem.* **115,** 567 (1936).

[2] The difference between the rate of activation of the "hydrolysable" and "nonhydrolysable" fractions is still better brought out when comparing the specific activity of the pyrophosphate, obtained from adenosintriphosphate after 7 min hydrolysis, to that the residual P, as found in a recent investigation the result of which will be published shortly.

reaction between inorganic phosphate ions and the hydrolysable esters is a very fast process. That the inorganic P present in the corpuscles does not reach exchange equilibrium with the plasma phosphate in the course of a few hours is due to the fact that a large part of the active phosphate ions are incorporated into the organic compounds of the corpuscles, while simultaneously non-active phosphate ions are freed to take place of the active ones and "dilute" the active inorganic phosphate

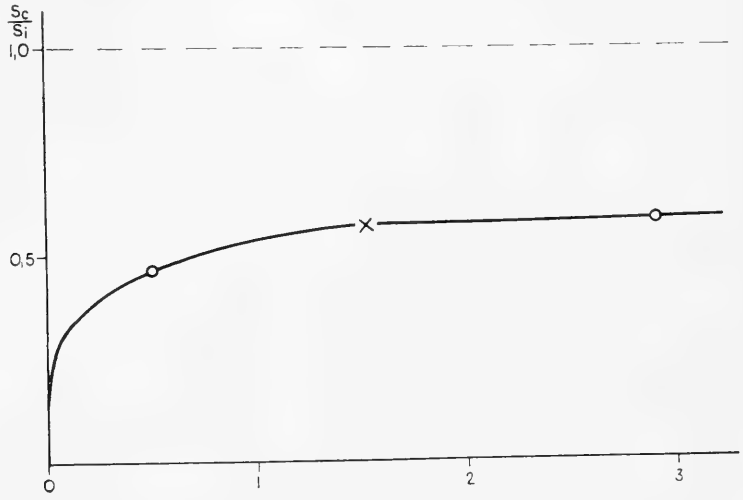

FIG. 1.

Ratio of the specific activities of the total acid- soluble phosphate and the inorganic phosphate present in the corpuscles.
× = Rabbit G. ○ = Rabbit H.

which penetrated into the corpuscles, diminishing thus the specific activity of the corpuscle inorganic P.

If the whole ester-phosphorus could exchange with the inorganic P, the activity-ratio should finally reach a value 1.0 (indicated by the dotted line). It is clear that the limiting value of $\frac{S_c}{S_i}$ is much lower, which is due to the presence of an organic phosphorus fraction in the corpuscles exchanging at a slow rate.

PENETRION OF PLASMA HPO_4 INTO CORPUSCLES

We may obtain information as to the rate of penetration of the $HPO^{''}$ from the plasma into the corpuscles by comparing, after a lapse of time, the specific activities of the plasma inorganic P and that of the total acid-soluble P present in the corpuscles. An alternative method would be to compare the specific activities of the plasma inorganic P and the

corpuscle inorganic P, but in this way the rate of penetration would be underestimated for the following reason : While active esters are forming in the corpuscle a corresponding amount of non-active P ester decomposes, producing non-active inorganic P which dilutes the active inorganic P present. (We denote as specific activity the activity per mgm P). Therefore, to arrive to a proper figure for the rate of penetration into the corpuscles we have to consider the total acid-soluble P present in the corpuscles. As already mentioned, one part of the organic P of the corpuscles reaches exchange equilibrium very rapidly while the other becomes activated at a slow rate ; and therefore the ratio of the specific activities of the corpuscle inorganic P and total acid-soluble P does not become = 1, but 1.8. This figure is only valid for the blood of the rabbit, while a different figure will presumably be obtained for blood containing appreciably more or less hydrolysable phosphorus. In the following discussion the calculation of the penetration of $HPO_4^{''}$, and of a magnitude which we will call a penetration coefficient and denote as a will be demonstrated (Comp. the list of symbols).

CALCULATION OF THE COEFFICIENT OF PENETRATION

The total activity of the plasma (a_p) is equal to the product of the specific activity (s_p) and the amount of phosphate (p) present in the plasma. (Definition of the specific activity.)

$$a_p = p \cdot s_p$$

and similarly for the corpuscles

$$a_c = c \cdot s_c$$

c denoting the total acid-soluble P of the corpuscles. During the exchange process $a_p + a_c$ remains constant, and therefore

$$- \Delta s_p = \frac{c}{p} \Delta s_c .$$

At the beginning of the experiment the specific activity of the corpuscles P was = 0 and, accordingly, when denoting the specific activities at the end of the experiment as S_p and S_c, we arrive at a value of the specific activity of the plasma phosphate at the beginning of the experiment

$$s_p = S_p + \frac{c}{p} S_c .$$

The rate of activation of the corpuscle P being proportional to $s_p - s_i$, we can write:

$$\frac{ds_c}{dt} = a(s_p - s_i)$$

As already mentioned, a is the coefficient of penetration. In an early stage of the experiment the change of s_i and s_p with time is linear and therefore, as far as we choose such experimental conditions that most of the activity is still to be found in the plasma, we arrive at the average value of s_p during the experiment

$$S_p + \tfrac{1}{2}\frac{c}{p} S_c$$

and in analogous way on the average

$$s_i = \tfrac{1}{2} S_i .$$

From which follows, considering that s_c increases linearly with time,

$$S_c = a\,(S_p + \tfrac{1}{2}\frac{c}{p} S_c - \tfrac{1}{2} S_i)\,t.$$

We found in our best experiment $\frac{c}{p} = 4$ and, as we saw that $S_i = 1.8\ S_c$ we conclude that the end value of the specific activity of the corpuscles total acid-soluble P when the experiment, as was in our case, is of re-restricted duration,

$$S_c = a(\,S_p + 1.1\,S_c)\,t.$$

The value of the penetration coefficient of the phosphate ions into the corpuscles, a magnitude we shall make use of in our later calculations, is given in Table 2.

TABLE 2. — CALCULATION OF THE PENETRATION-COEFFICIENT OF PHOSPHATE-IONS (a) INTO CORPUSCLES

S_p denotes the specific activity of the total acid-soluble P in the corpuscles; S_p that of the plasma at the end of the experiment

	Time in min.	$\left(\frac{S_c}{S_p}\right)$	a
Rabbit C	170	0.19	0.0009
	170	0.20	0.0010
Rabbit G	90	0.14	0.0014
	90	0.21	0.0019
Average value		—	0.0013
Average value (Rabbit C alone)		—	0.0010

In what follows we shall compare the rate of interpenetration of labelled phophate ions into the corpuscles with that of labelled hexose-monophosphate molecules. As the latter are easily decomposed in the plasma, we have first to discuss the behaviour of the hexose-monophosphate in this medium.

RATE OF DECOMPOSITION OF HEXOSE-MONOPHOSPHATE

The usual method applied to the study of the decomposition of hexose-monophosphate under the action of enzymes is the determination of the amount of phosphate ions split off in the course of the experiment. This is a highly satisfactory method if, at the start of the experiment, no appreciable amount of inorganic phosphate is present. It is not very satisfactory, however, if the decomposition of a slight amount of phosphorus ester is to be determined in the presence of large amounts of inorganic phosphate. In such a case the use of labelled hexose-monophosphate and the determination of the amount of radioactive phosphate split off is much to be preferred to the first mentioned method. Even large amounts of inorganic phosphate present at the start of the experiment will in no way influence the results as these are, in contrary to the phosphate split off from the labelled phosphorus ester, not radioactive, and thus not recorded by activity measurements.

In our experiments we have shaken 10 cc. of rabbits' blood for a few hours in a thermostat, after the addition of labelled hexosemonophosphate containing about $^1/_{15}$ mgm P. The hexosemonophosphate (Embden ester) was prepared by Dr Ostern and kindly presented us by Professor Parnas. At the end of the experiment the activity of the total acid-soluble P of the corpuscles, that of the organic acid-soluble P of the plasma, and also that of the inorganic phosphate present in the plasma was determined. The results obtained are seen in Table 3.

The first two samples were shaken in air after the addition of oxalate, the four latter samples in a mixture of CO_2 and O_2 after addition of

Table 3. — Hydrolysis of Labelled Hexosemonophosphate Added to Blood in vitro at 37°

	Time in min	Fraction decomposed	Velocity constant of decomposition in min^{-1}
Rabbit A	150	0.26	0.0021
	150	0.37	0.0031
Rabbit C	80	0.15	0.0021
	80	0.15	0.0020
	170	0.29	0.0020
	170	0.32	0.0023

heparin. The velocity constant was calculated by making use of the equation valid for mono-molecular reactions

$$\ln \frac{A_0}{A_t} = kt,$$

A_t being the total activity of the ester present in the blood at the time t, A_0 at the beginning of the experiment. It follows from the fair constancy shown by the velocity constant recorded in Table 2 that in the hydrolysis of the hexosemonophosphate equilibrium is far from being reached in three hours. It is of interest to compare the velocity constant of the hydrolysis of the labelled hexosemonophosphate which we obtained under the action of enzymes present in the blood with the value ROBISON[1] found when hydrolysing the ester by 0.1 n H_2SO_4. The acid was found to be much less effective in hydrolysing hexosemonophosphate than the enzymes, the value of $k = 2.2 \times 10^{-6}$ min^{-1} being found by him.

In one case labelled hexosemonophosphate was added to plasma and we found, after the lapse of 175 min, 23% of the ester to be decomposed, thus approximately the same amount which decomposes during the same time in the presence of blood corpuscles (31%).

When labelled ester disappears we have to distinguish between two possibilities, in one case the number of ester molecules actually diminishes, in the other case the active phosphorus atom present in the ester molecules is replaced through an enzymatic exchange process by a non-radioactive one and no change in the number of ester molecules occurs. That in the above discussed cases we have to deal with the first mentioned possibility (decomposition of the ester) follows from the experiment to be described. To 10 cc. plasma non-active hexosemonophosphate containing 0.15 mgm P and radioactive sodium phosphate of negligible weight was added and after the lapse of 170 min the activity of the plasma esters determined. If the loss of activity by the active ester in the experiments recorded in Table 3 should be due to an exchange process we would have to expect about 5% of the activity of the sodium posphate to be incorporated in the originally non-active hexosemonophosphate added to the plasma. The result of our experiment, however, has shown that the activity of the added hexosemonophosphate isolated together with the other minute amounts of esters present in the plasma amounted to less than $^1/_{10}$ of the calculated activity. We have therefore to conclude that at least 90% of the activity loss of the labelled hexosemonophosphate recorded on Table 3 is actually due to decomposition and not to an exchange process.

[1] R. ROBISON, *Biochem. J.* **27**, 2191 (1932).

UPTAKE OF HEXOSEMONOPHOSPHATE BY THE BLOOD CORPUSCLES

We interpreted the formation of active phosphorus esters found in the corpuscles as due to an enzymatic exchange process inside the corpuscles, (the number of ester molecules decomposed being presumably replaced by an equal number of newly formed molecules) into which some of the active inorganic phosphate added to the plasma penetrated. An alternative explanation would be that active phosphorus ester molecules are formed in the plasma, diffuse into the corpuscles and are replaced, in experiments *in vitro*, by an equal number of non-active molecules leaving the corpuscles. We can test the correctness of this explanation for each compound by adding to the plasma the active phosphorus ester and determining, after the lapse of few hours, the activity of the phosphorus ester molecules and the inorganic phosphate of the corpuscles. So far we only carried out such experiments with active hexosemonophosphate, prepared by Dr. OSTERN and presented to us most kindly by Professor PARNAS. Several of the other labelled phosphorus ester compounds were also synthesized in the laboratory of the latter. The result obtained by us is that, if hexosemonophosphate molecules diffuse at all into the corpuscles, the rate of their penetration must be much slower than that of the phosphate ions. We arrived at this result by supposing that the amount of active P to be expected in corpuscles after the lapse of a certain time is wholly due to the penetration of active phosphate ions from the plasma into the corpuscles and independent of the presence of active hexosemonophosphate in the plasma. The next step is to compare the calculated values for the activity of the corpuscles P with those found by the experiment and to ascertain if any difference is shown by the two values. Should that not be the case, then we must conclude that the rate of penetration of the hexosemonophosphate molecules into the plasma is negligible compared with that of the phosphate ions. The amount of labelled P in the corpuscles is zero at the start, i. e. after addition of active hexosemonophosphate to the plasma, and increases with time as discussed on p. 477. As in the course of the experiment only a small part of the active hexosemonophosphate is hydrolysed, we are entitled to make the simplifying assumption that the increase of the specific activity of plasma inorganic P takes place in a linear fashion. We also assume that the decrease of the difference in the specific activities of plasma phosphate P and corpuscle phosphate P with increasing time will also take place according to a linear function[1]. The average value (comp. 474) of the last mentioned difference will be

$$\tfrac{1}{2}(S_p - S_i) = 0.5\,S_p - 0.9\,S_c$$

[1] This assumption though incorrect does not cause an appreciable error.

and the specific activity of the corpuscles P at the end of the experiment

$$S_c = a\,(0.5\,S_p - 0.9\,S_c)\,t\,.$$

The value of a being known ($a = 0.0010$), the quantity $\frac{S_c}{S_p}$ can be evaluated. The figures thus obtained and also those supplied by the experiment are recorded in Table 4.

TABLE 4

	Time of experiment in min	Values of $\frac{S_c}{S_p}$	
		calculated	found
Rabbit C	80	0.04	0.03
	80	0.04	0.04
	170	0.07	0.07
	170	0.07	0.09

UPTAKE OF PHOSPHATE IONS BY THE BLOOD CORPUSCLES IN EXPERIMENTS IN VIVO

The interpretation of the results of experiments *in vivo* is much complicated by the fact that active phosphate introduced into the blood stream is rapidly exchanged with phosphate of bone tissue. Such an interaction leads to a very rapid decrease in the activity of the blood after intravenous administration of active phosphate as the specific activity of the plasma inorganic P at the end of the experiment differs very strongly from the value prevailing on the average during the experiment. To facilitate the interpretation of the result the labelled sodium phosphate

TABLE 5. — PERCENTAGE OF THE TOTAL LABELLED P INJECTED DURING THE COURSE OF THE EXPERIMENT PRESENT IN THE CIRCULATION

(Labelled sodium phosphate administered drop by drop in the course of three hours)

Time in minutes	Percentage of the total P administered in the course of 3 hours present in circulation
18	1.16
61	2.64
117	4.00
168	5.36

was injected into the ear vein of a rabbit drop by drop in the course of the experiment which took 3 hours. During the experiment small blood samples (0.1 — 0.3 mgm) were taken from the vein of the other ear of the rabbit and their activity determined. The results of the experiment are seen in Tables 5 and 6.

TABLE 6. — SPECIFIC ACTIVITIES OBTAINED

Labelled P injected drop by drop during the course of the experiment (3 hours)

Fraction	Relative specific activity
Plasma inorganic P	100.0
Corpuscle inorganic P	11.0
Corpuscle acid-soluble organic P	7.7
Marrow inorganic P	7.5
Muscle inorganic P	6.9
Muscle creatine P	0.95
Muscle acid-soluble after removal of inorganic creatine P	0.78
Tibia diaphysis P average	0.27

As seen from the figures of Table 5 the specific activity of the plasma inorganic P increases during the experiment somewhat slower than proportional to time. If the increase of the specific activity would be proportional to the time of the experiment the average specific activity of the plasma inorganic P, which we wish to know, should be $= \frac{1}{2} S_p$. This can be considered to be a lower limit of the average specific activity. To arrive at an upper limit we could assume the average specific activity to be equal to the maximum value observed, which is S_p. Making the assumption $s_p = \text{prop.} \sqrt{t}$, we arrive at the average value of the specific activity of the plasma inorganic P $= \frac{1}{2} S_p$. The value for the specific activity of the total acid-soluble corpuscle P (comp. p. 477) is approximately given by the formula

$$S_c = \tfrac{2}{3} a\, t\, (S_p - 1.8\, S_c)\,.$$

For the ratio $\frac{S_c}{S_p}$, after the lapse of 175 min., we arrive at the figure 0.12 (using the value of a found in our experiments *in vitro*), while the experimental value of the specific activity of the acid-soluble P of the corpuscles (comp. Table 6), after the lapse of 175 min., was found to be 8% of the plasma inorganic P at the same time, or $\frac{S_c}{S_p} = 0.08$. The calculated and experimentally found values of the rate of labelling of

the corpuscles acid-soluble P are thus in good agreement. We arrived at the calculated figure by making use of the a value obtained with rabbits' blood in experiments *in vitro* (taking $a = 0.0013$ for rabbit I).[1]

ADMINISTRATION OF LABELLED HEXOSEMONOPHOSPHATE

Labelled hexosemonophosphate containing 6 mgm. P was administered to a rabbit weighing 2.7 kgm. by intravanous injection. After the lapse of $1\frac{1}{2}$ hours the animal was killed and the specific activity of the fractions recorded in Table 5 determined.

When interpreting the above figures we must bear in mind that the greatest part of labelled inorganic P formed through the decomposition of active hexosemonophosphate enters the tissues. That the specific activity of the plasma inorganic P is, in spite of this fact, higher than that of the plasma ester P clearly indicates that a very large part of the active hexosemonophosphate injected into the blood must have been decomposed in the course of the experiment. We must also take in consideration that besides the 4 mgm.% hexosemonophosphate P added the plasma contained also its normal ester P content of about 0.5 mgm.% which became partly labelled, this being made possibly through the presence of labelled inorganic P formed through the decomposition of active hexosemonophosphate. But even disregarding the presence of esters other than hexosemonophosphate, by comparing the activity of the hexosemonophosphate introduced with that of the total plasma ester present, after the lapse of $1\frac{1}{2}$ hours, we arrived at the result that more than 99.9% of the labelled hexosemonophosphate administered left the circulation within $1\frac{1}{2}$ hours. In making the above calculation we assumed the blood content of the rabbit to amount to 160 cc.

In our *in vitro* experiments $^1/_{15}$ mgm. of hexosemonophosphate was added to 10 cc. blood, thus the concentration of the latter was about $\frac{1}{6}$ of that in the experiment *in vivo*.[1] While in the *in vitro* experiment in the course of $2\frac{1}{2}$ hours $\frac{1}{5}$ of the labelled hexosemonophosphate was decomposed, in the *in vivo* experiment in the course of $1\frac{1}{2}$ hours more

[1] When isolating the inorganic P and the different fractions of the ester P present in the corpuscles and comparing their specific activity in experiments *in vitro* and *in vivo* some differences were found. The results of these experiments will be shortly published.

than 99.9% was removed[1]. A powerful agency producing hydrolysis of hexosemonophosphate is ROBISON's bone enzyme. The presence of small amounts of this enzyme in the plasma was found by MARTLAND and ROBISON[2] which according to them is possibly derived from bone by slow diffusion. These small amounts of bone enzymes were presumably responsible for the hydrolysis of hexosemonophosphate in our experiments *in vitro*, while the much more rapid disappearance of the labelled hexosemonophosphate from the blood *in vivo* is probably due to the much larger amounts of bone enzyme present in the bones and other organs, especially the kidneys[3]. As seen in Table 7 the specific activity of the corpuscle inorganic P is appreciably lower than that of the plasma inorganic P, while that of the corpuscle ester P is only slightly lower than that of the corpuscle inorganic P.

TABLE 7. — SPECIFIC ACTIVITY OF P FRACTIONS 1½ HOURS AFTER ADMINISTRATION OF LABELLED HEXOSEMONOPHOSPHATE

Fraction	Specific activity
Plasma inorganic P	1
Plasma ester P	0.9
Corpuscles inorganic P	0.3
Corpuscles ester P	0.23
Liver inorganic P[4]	0.62
Liver ester P	0.22

[1] A large part of the hexosemonophosphate removed may have been taken up by the tissues. R. T. BRAIN, H. O. KAY and P. G. MARSHALL (*loc. cit.*) found namely that in the course of 5 min more than three-quarters of the injected glycerophosphate left the human circulation and as in this time it had not been excreted in the urine, nor hydrolysed into inorganic P in the blood, it must have been taken up by the tissue.

[2] MARTLAND and ROBISON, *Biochem. J.* **20,** 847 (1926).

[3] BODANSKY [*J. Biol. Chem.* **118,** 391 (1937)] concludes that the phosphatase in question comes from the bone, kidney or some other tissue, but not from the intestinal mucose.

[4] We were prevented in extracting at once the inorganic and acid soluble organic P of the liver and therefore some of the latter may have been decomposed (comp. E. JAKOBSEN, *Biochem. Z.* **242.** 232, (1931), supplying inorganic P of low activity. The specific activity of the inorganic P present as such in the intact liver may therefore have been higher than stated above. In this connection it is of interest to know that in the case of a rat, which had been injected with labelled hexosephosphate and killed 2 hours later, no such difference was found. Relative specific activities: Plasma inorganic phosphate 1.0; liver inorganic phosphate 0.97; liver ester 0.58.

ON THE ORIGIN OF PHOSPHATIDES OF PLASMA AND CORPUSCLES

We have already mentioned that the amount of labelled phosphatides formed in the blood within a few hours is entirely negligible compared to that of labelled acid-soluble organic compounds formed. The phosphatides present in the plasma are released by the organs in which phosphatides are synthesized, primarily by the liver, but also by the intestinal mucosa, and possibly by other organs. The synthesis of phosphatides in the different organs was investigated in recent years using fatty acids, which could be traced by chemical analysis[1], and also by the use of radioactive P as an indicator[2]. In several cases the change in the degree of saturation of the fatty acid component of the phosphatides extracted from the intestinal mucosa, liver, etc., was studied after feeding cod liver oil which contains a large amount of unsaturated fats. Within a short time an increase in the iodine number of the fatty acids was found. For example, within 2 days after the change of diet the iodine number of phosphatides of the intestinal mucosa increased from 93 to 160. From this result it follows that within 2 days an appreciable amount of the phosphatide molecules present in the intestinal mucosa was renewed. Instead of feeding a mixture of fatty acids showing a different degree of saturation and having a high average iodine number, SINCLAIR fed, in his later experiments, fats containing pure (85%) elaidic acid, a geometric isomer of oleic acid, an easily traceable substance, since it forms a lead salt which is insoluble in ether, differing in this regard from all other unsaturated acids. 8 hours after feeding elaidic acid to cats, 15% of the fatty acids extracted from the plasma phosphatides were found to contain elaidic acid, while hardly any was found in the phosphatides obtained from the corpuscles. This result may be possibly interpreted as a proof of lack of a phosphatide turnover in the corpuscles. Another chemical indicator used to follow up the turnover of phosphatides is iodised fat which was fed by ARTOM to animals. Also in this case the presence of iodised fatty acid in the phosphatide molecule is a proof of their formation after the administration of iodised fat. By using this method ARTOM found the presence of iodised fatty acids in the phosphatides extracted not only from the plasma but also from the corpuscles. In the latter the concentration of the iodised fat was

[1] Comp. R. G. SINCLAIR's *Physiol. Rev.* **14,** 351 (1934). The papers of the same author and collaborators, *J. Biochem.* **115,** 211 (1936); **118,** 122 (1937); **121,** 361 (1937); C. ARTOM, *Arch. int. Physiol.* **36,** 101 (1933).

[2] C. ARTOM, C. PERRIER, M. SANTANGELLO, G. SARZANA and E. SEGRÉ, *Arch. Int. Physiol.* **45,** 32 (1937); **47,** 245 (1938); L. HAHN and G. HEVESY, *Skand. Arch. Physiol.* **77,** 148 (1937); G. HEVESY and E. LUNDSGAARD, *Nature* **140,** 275 (1937). B. A. FRIES, S. RUBEN, I. PERLMAN and I. C. CHAIKOFF, *J. Biol. Chem.* **122,** 169 (1937); **123,** 587 (1938).

even higher (3.3% of the total fatty acids) than in the former (2.0%), a still lower content being found in the phosphatide fatty acids secured from the liver. The result obtained as to the turnover of phosphatides in the corpuscles, when using the elaidic acid method, is thus just the opposite of that arrived at when applying iodised fat as an indicator. While these indicators proved to be very useful to show that a rapid turnover of phosphatides actually takes place in some of the organs they are less adapted to permit conclusions of a quantitative nature to be drawn.

By introducing elaidic acid or iodised fat into the phosphatide molecule the properties of the latter are appreciably changed and not only will the different organs utilise the above mentioned substances in the formation of phosphatides only to a restricted extent, but the rate of uptake of these compounds may differ for different organs. In rats provided with large amounts of elaidic acid throughout the entire period of prenatal and postnatal development, the elaidic acid content of the fatty acids in the phosphatides of the brain was found to be only $\frac{1}{4}$ of that of the liver and muscles. If one finds a slower turnover of elaidic acid in the brain phosphatide than in other organs, this result can be partly due to a slower phosphatide turnover in the brain, and partly to a greater degree of selection in the building up of phosphatides in the brain than in the liver. The rate of the incorporation of elaidic acid into the phosphatide molecules will therefore fail to be a quantitative measure of the rate of phosphatide rejuvenation in the brain tissue, though this method revealed much important information as, for example, that the phosphatide turnover in the muscles is much slower than that of the liver and the intestinal mucosa. In rats, the incorporation of elaidic acid into the liver phosphatides was found to be essentially completed within one day but in the muscle transformation had occurred only after a period of many days. One of the great advantages of the application of isotopic indicators is that the replacement of ^{31}P by ^{32}P for example in the phosphatide molecule does not change the chemical character of the substance to any noticeable extent and therefore any possible preference of an organ for the ^{32}P phosphatide can be disregarded. A quantitative comparison of the phosphatide turnover in different organs by using ^{32}P as an indicator was carried out by different experimentors, a slow turnover being found in the brain and muscles, a fast one in the milk gland, the liver, the kidneys and the intestinal mucosa, while we find a fairly fast rate in tumor tissue. Taking the specific activity of the phosphatide P extracted from the liver of a mouse to be 100, we find for the specific activity of that extracted from the muscles and the graft of the brest tumor 18 and 9% respectively. These figures indicate the relative rate of resynthesis of the phosphatide molecules. They were obtained by extracting and analysing the phosphatides from the organs,

4 hours after the administration of labelled sodium phosphate. These figures, however, fail to inform us as to the percentage of the phosphatide molecules which were renewed within the last 4 hours. Most of the active phosphate ions will exchange with bone and other tissue phosphate and will thus be prevented from taking part in the synthesis of phosphatide molecules. Knowledge relative to the percentage of, for example the liver phosphatides, renewed within 4 hours can be obtained, as already discussed on p. 485, by administering the sodium phosphate solution drop by drop and thus keeping the active phosphate concentration of the blood at an approximately constant level, or in perfusion experiments carried out on the isolated liver. In the latter case, active sodium phosphate is added to the blood circulating through an isolated liver and, after the lapse of a few hours, the specific activity of the plasma inorganic P and that of the phophatide P extracted from the liver are compared. Through the kindness of Prof. LUNDSGAARD we were able to carry out such a determination from which it was concluded that, in the course of $2\frac{1}{2}$ hours, about 2% of the phosphatides in the cat liver are renewed[1]. In the same time, when blood is shaken with active sodium phosphate, only a very slight amount of active phosphatide was formed. From these experiments it may be concluded that less than 0.1% of the phosphatide molecules present in the isolated blood was renewed in the course of $2\frac{1}{2}$ hours. In experiments on goats, 4 hours after injecting labelled sodium phosphate, less and probably much less than 1% of the blood phosphatide molecules were labelled. We have therefore to conclude that the phosphatide molecules present in the blood can only get rejuvenated by an influx of molecules from the organs like the liver in which they were synthesized. As a possible source of formation of the blood phosphatides the liver is first to be considered in view of the fast phosphatide turnover found in the liver and the large amounts of phosphatides stored in it. In this connection the results obtained by NEDSWEDSKY[2] should be recalled according to which blood leaving the liver contains 23% more phosphatide than that entering the liver from the portal vein.

Laying hens are especially well suited to the study of phosphatide metabolism. A hen laying daily incorporates into the yolk 1—2 gm of phosphatides corresponding to about 60 mgm of P. We found that these phosphatides were not produced in the ovary but were carried by the plasma in the main from the liver. To what extent phosphatides are carried into the circulation through the lymph from the intestinal mucosa is not yet settled. In the above mentioned experiment the specific activity of the phosphatide P extracted from the hens' intestinal mucosa

(1) L. A. HAHN and G. C. HEVESY, *Biochem. J.* **32,** 342 (1938).
(1) S. W. NEDSWEDSKY and K. ALEXANDRY, *Z. physiol. Chem.* **119,** 619 (1928).

was found to be only $\frac{1}{4}$ of that of the plasma phosphatide P. The greatest part of the active phosphatide molecules present in the plasma of the hen could therefore not originate from the intestinal mucosa but must have been formed in the liver and, possibly to minor extent, in other organs. As the plasma of the hen contains only about 20 mgm % phosphatide P, thus only $\frac{1}{3}$ of that incorporated in the yolks daily, over $\frac{9}{10}$ of the plasma phosphatides of the blood is removed from the latter, in the course of a day, and replaced by newly formed molecules. If the plasma phosphatides originate from the liver, after the lapse of a day, the specific activity of the plasma phosphatide P should no longer differ materially from that of the liver phosphatide P. In an experiment, in which the hen was killed 28 hours after injecting the labelled phosphate, the plasma phosphatide P showed a specific activity amounting to 82% of that of the liver phosphatide P. When interpreting the low figure found for the phosphatide turnover in the intestinal wall of the hen compared with that found in the liver we have, however, to bear in mind that the labelled inorganic phosphate reaches the digestive tract at a later and thus more "diluted" (with inactive phosphate) state than the liver. The active phosphate injected will be promptly carried to the liver while it enters the intestine only in the form of saliva, gastric juice, bile and pancreatic juice and, possibly to some extent, through the intestinal wall[1] in the digestive tract. Therefore, when comparing the specific activities of the liver phosphatide P with that of the intestinal mucosa phosphatide P after injection of active phosphate we are apt to overestimate the phosphatide turnover of the liver while, when feeding the active sodium-phosphate, we must expect the opposite to be the case.

A comparison of the specific activity of the liver inorganic P with that of the liver phosphatide P leads to the result that, after the lapse of 28 hours, the former was about two times greater than the latter. Thus only less than half of the phosphatide molecules present in the hen's liver was newly formed within that time, the rate of regeneration of the tissue phosphatides being thus a comparatively slow process. In experiments we carried out on human subjects, in the course of a day less than 30% of the plasma phosphatides was renewed and a rough estimate, taking into account the change of the spec. activity of the phosphate with time, indicates that after the lapse of a week this fraction is still less than one-half (comp. Table 8).

We have already mentioned that *in vitro* experiments have shown that in the blood only a minimal new formation of phosphatide molecules takes place; the phosphatides present in the corpuscles must therefore have been incorporated in the latter during their formation,

[1] G. F. Youngyurg, *Proc. Exp. Physiol. Med.* **36,** 230 (1932).

or alternatively diffused from the plasma into the corpuscles[1]. As already mentioned, within a day, the plasma phosphatides of a daily laying hen were replaced up to 82%, by phosphatide molecules carried into the circulation from the liver and other organs. In spite of this thoroughgoing replacement of the plasma phosphatides, the phosphatide molecules present in the corpuscles are renewed only to an extent equal to $\frac{1}{3}$ of that of the plasma phosphatides in the course of 28 hours. In human subjects, after the lapse of one day, the corpuscle phosphatide shows a specific activity amounting to only $\frac{1}{5}$ or less of that of the plasma phosphatide P and even after the lapse of 8 days the ratio is still $\frac{1}{2}$. When the corpuscles are formed from plasma containing labelled phosphatides they are bound to contain such. As seen above, even after the lapse of a week the labelled fraction of the plasma phosphatides is still twice as high as that of the phosphatides present in the corpuscles. From this result we can conclude that less than half of the corpuscles are produced in a week. One could object to the above conclusions on the ground that the phosphatides are composed of different constituents, lecithin, cephalin and so on, each of which may contain very different fatty acids and, if those components which are mainly represented in the corpuscles are renewed at a slower rate than those chiefly found in the plasma, this difference may also tend towards a low value for the specific activity of the corpuscle phosphatide P. In human blood, the composition of the corpuscle phosphatides was found to be not very different from that of the plasma phosphatides[2] and as we have found that, after a lapse of a day, the specific activity of the corpuscle phosphatides is only 8—19% of that of the plasma phosphatides (comp. Table 8) we are justified in concluding that at least the most active component of the phosphatide mixture of the corpuscles must show a lower specific activity than its counterpart in the plasma.

This fact definitely excludes the possibility that the active phosphatides are formed in the corpuscles and diffuse into the plasma. The result discussed on page 486, according to which the corpuscle phosphatides contain more iodised fat than the plasma phosphatides, is presumably to be explained by a greater preference of the corpuscles for phosphatides containing an iodised fatty acid component.

[1] An investigation carried out recently in collaboration with L. Hahn lead to the result that a fairly slow exchange between a part of the phosphatide molecules present in the corpuscles and those present in the plasma takes place. In the course of 4 hours about 5% of the phosphatides present in the corpuscles were exchanged.

[2] E. Kirk, *J. Biol. Chem.* **123,** 637 (1938). According to this experimentor plasma phosphatide contains as an average 13% lecithin, 47% cephalin, and 40% sphingomyelin, while the corresponding figures for the corpuscles are 16, 60 and 24.

TABLE 8. — PARTS IN MILLION OF THE TOTAL ACTIVITY INJECTED SUBCUTANIOUSLY PRESENT IN 1 MGM P EXTRACTED FROM THE PLASMA AND CORPUSCLES OF HUMAN SUBJECTS

a) Boy	
Sample taken after 120 min	
Plasma phosphate	201
Corpuscle total acid soluble P	57
Sample taken after 7 days	
Plasma phosphate	10
Plasma phosphatides[1]	14
Corpuscle phosphatides	6
b) Young female	
Sample taken after 130 min	
Plasma phosphate	480
Corpuscles inorganic phosphate + hydrolysable ester P (hydrolysed for 7 min at 100° in 1 n HCl)	87
Corpuscles non-hydrolysable ester P	62
Sample taken after 1 day	
Plasma phosphate	105
Corpuscles total acid soluble	173
Plasma phosphatide	31
Corpuscles phosphatide	2.3
c) Old female	
Sample taken after 140 min	
Plasma phosphate	224
Corpuscles inorganic phosphate + hydrolysable ester P (hydrolysed for 7 min at 100° in 1 n HCl)	61
Corpuscles non-hydrolysable ester	34
Sample taken after 1 day	
Plasma phosphatide	7.5
Corpuscles phosphatide	1.3
Sample taken after 8 days	
Plasma phosphate	10
Plasma phosphatide[(1)]	16
Corpuscles phosphatide	7

[(1)] We witness here an example of the case discussed in detail in former papers (for example HAHN and HEVESY, *Skand. Arch. Phys.* **77,** 148 (1937) in which, due to the rapid exchange between bone phosphate and plasma phosphate the active inorganic P is renewed from the plasma at a rapid rate while the removal of the labelled phosphatide P is a much slower one. Some of the phosphatide molecules were formed from a highly active plasma and were still present after a lapse of 7 days, while the greater part of the inorganic P disappeared at this time from the plasma. Similar cases can also be found when investigating the acid soluble P.

Summary

The rate at which labelled phosphate ions added to the plasma of rabbit blood penetrate into the corpuscles was determined. A comparison of the specific activity (activity per mgm P) of the inorganic P and of the ester P extracted from the corpuscles suggests that the penetration of the labelled inorganic phosphate into the corpuscles is a comparatively slow process while the process in which the labelled inorganic phosphate is incorporated into the easily hydrolysable organic compounds of the corpuscles is a fast one.

The rate of increase of the specific activity of the total acid soluble phosphorus (s_c) present in the corpuscles with time (t) is proportional to the difference in the specific activity of the inorganic P of the plasma (s_p) and that of the corpuscles (s_i).

$$\frac{ds_c}{dt} = \alpha (s_p - s_i).$$

The proportionality factor of the above equation (α), denoted as coefficient of penetration, was calculated; the latter was found to be about the same in experiments *in vitro* and *in vivo*.

By making use of labelled sodium hexosemonophosphate it was found that, when shaking the latter with rabbit blood, $^1/_5$ hydrolyses within $2^1/_2$ hours. From sodium hexosemonophosphate administered, by intravenous injection, to a rabbit after the lapse of $1^1/_2$ hours less than $^1/_{10}\%$ was left in the circulation.

By comparing the rate of formation of labelled ester P in the corpuscles after introducing in one case labelled hexosemonophosphate in the other labelled inorganic phosphate, we arrive at the result that the labelled phosphate ions which penetrate into the corpuscles are utilised for the formation of labelled ester molecules. If any labelled hexosemonophosphate penetrates into the corpuscle at all, the rate of its penetration is much lower than that of the phosphate ions.

The rate of renewal of the phosphatide molecules present in the blood was found to be very much slower than that of the acid-soluble compounds. The synthesis of the former was found to take place in the organs from which they are carried into the circulation. The replacement of the corpuscle phosphatides is a much slower process than that of the phosphatides present in the plasma, the ratio in human blood after one day being 6 : 1, after a week 2 : 1.

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50. RATE OF PENETRATION OF IONS INTO ERYTHROCYTES

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The hindrance which an ion encounters when passing the phase boundary can be measured by measuring the rate at which labelled and non-labelled ions interchange through the phase boundary. When one phase contains ^{23}Na ions, the other phase besides ^{23}Na ions also ^{24}Na ions of negligible weight, we obtain by determining the rate at which ^{24}Na penetrates into the second phase a correct measure of the hindrance which sodium ions encounter when passing the phase boundary and, thus, a correct measure of the permeability of the phase boundary to sodium ions if only we perform the experiment in such a way that the amount of ^{24}Na returning from the second phase into the first phase can be disregarded. In this paper, the rate of interchange of phosphate, chloride, and sodium between plasma and corpuscles is discussed.

PENETRATION OF LABELLED PHOSPHATE INTO THE CORPUSCLES OF THE RABBIT

We extended our former measurements (Aten and Hevesy 1938, 1939; Hahn and Hevesy, 1938, 1940) by further experiments on the penetration of labelled phosphate into the corpuscles. The distribution of ^{32}P between corpuscles and plasma of equal weight is seen in Fig. 1. In these experiments, the ^{32}P was added as sodium phosphate of negligible weight to rabbit blood. The blood to which ammonium oxalate or heparin was added was saturated with a mixture containing 5 p.c. CO_2 and 95 p.c. O_2 and shaken in the thermostat kept at 37. Corpuscles and plasma were separated by centrifuging sharply and the activity of the fractions obtained was compared. We omitted washing of the corpuscles, since such a procedure may lead to a removal of some of the ^{32}P present in the corpuscles.

To determine the amount of plasma adhering to the centrifuged corpuscles we washed, in test experiments, the corpuscles thoroughly with a physiological sodium chloride solution and determined the nitrogen

content of the sodium chloride solution and of a plasma sample by Kjeldahl's method. From the figures obtained the amount of plasma present in the sodium chloride solution was calculated to be 3 per cent of the plasma content of the blood. This is the upper limit of the percentage plasma adhering to the centrifuged corpuscles, since some corpuscle

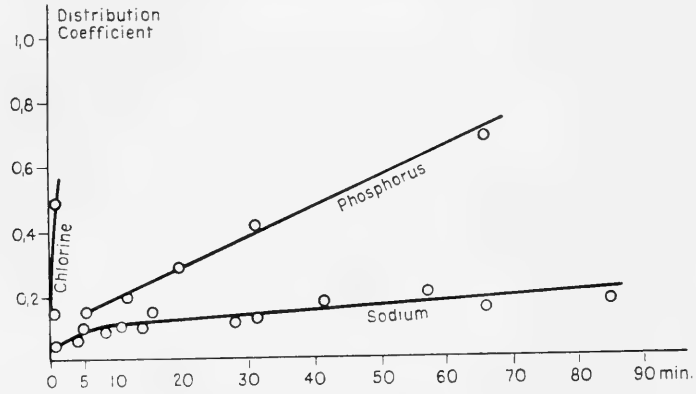

FIG. 1. Distribution of labelled ions between corpuscles and plasma of equal weight at 37°. (Rabbit blood was used in the experiments with chloride and phosphate, dog blood[1] in the experiments with sodium. The ordinate indicates the distribution coefficient of the labelled ion between corpuscles and plasma of equal weight.)

nitrogen may have leaked into the sodium chloride solution during the washing procedure.

Another method to test the amount of plasma adhering to the corpuscles is the following. Labelled phosphate is added to blood kept at 0° and the blood sample is centrifuged at once. When plasma adheres to the corpuscles the corresponding amount of labelled phosphate will be found in the corpuscle fraction. By this method, the corpuscle fraction was found to contain 3 per cent of the plasma. This value indicates the upper limit of the amount of adhering plasma, since some of the ^{32}P found in the corpuscle fraction may be due to a replacement of a part of the non-labelled phosphate ions adsorbed on the surface of the corpuscles by labelled ions.

That the line seen in Fig. 1 does not start at 0 is due partly to the presence of some plasma containing ^{32}P in the corpuscle fraction and presumably partly to the presence of some labelled phosphate adsorbed from the plasma by the surface of the corpuscles. In view of the very extended corpuscle surface, it is conceivable that non-negligible amounts

[1] ^{32}P added to dogs blood kept at 37° was found to be almost equally distributed after the lapse of 2 hours between plasma and corpuscles of equal weight.

of the plasma constituents get adsorbed on the surface of the corpuscles and a very short time may suffice to replace adsorbed non-labelled phosphate ions by labelled ones.

Furthermore, it is seen in Fig. 1 that the rate of penetration of the phosphate ions into the corpuscles is a fairly slow process. After the lapse of 1 hour, 1 gm of plasma contains about twice as much ^{32}P atoms as 1 gm of corpuscle. From 100 ^{32}P atoms added to the blood, about 37 are found in the corpuscles, and 63 in the plasma.

EXPERIMENTS OF LONG DURATION

Since the composition of drawn blood changes on standing, it is not advisable to carry out experiments with drawn blood lasting more than a few hours.

Information about the amount of ^{32}P which penetrates into the corpuscles in the course of several hours or days can be obtained through experiments carried out *in vivo* (Aten and Hevesy 1939). The results of such experiments are seen in Table 1. The specific activity stated is the ratio of the ^{32}P and the total P content of the sample. The specific activity of the plasma inorganic P is taken to be 100.

In the case of a complete interchange between plasma inorganic P and corpuscle acid soluble P these P fractions should have the same specific activity. If the ratio of the specific activity of the inorganic P of the plasma and the P of the corpuscle is, for example, 10, then every tenth acid soluble corpuscle P atom got replaced by P atoms present in the plasma at the start of the experiment. In all these experiments, the activity level of the plasma was kept approximately constant. This state was obtained by injecting labelled phosphate to the rabbit throughout the experiment.

In the first phase of the experiment, the rate of accumulation of ^{32}P in the corpuscles is slower than the rate of incorporation of ^{32}P into the easily hydrolysable organic P compounds which takes place inside the corpuscles. The rate of formation of active, easily hydrolysable P compounds is, thus, regulated by the speed of intrusion of ^{32}P into the corpuscles. The renewal of some of the "non-hydrolysable" organic P compounds takes place at a comparatively slow rate, and the rate of formation of active molecules of such compounds is not regulated by the influx of ^{32}P into the corpuscles but by the speed of new formation of these "non-hydrolysable" acid soluble P molecules. After the lapse of 9 days, we still find in the corpuscles not renewed acid soluble P molecules, while practically none are present after the lapse of 50 days. During this interval, the major part of the corpuscles has also been renewed.

PENETRATION OF ^{32}P INTO THE CORPUSCLES OF HUMAN SUBJECTS

Labelled phosphate was found to penetrate at a similar rate into the corpuscles of human as into the corpuscles of rabbit blood. In two experiments carried out *in vitro* at 37°, after the lapse of 95 minutes, 29.2 and 31.7 percent of the labelled phosphate ions added to the blood were found to be in the corpuscles.

TABLE 1. — PENETRATION OF LABELLED P INTO THE CORPUSCLES OF THE RABBIT IN EXPERIMENTS IN VIVO

Administration of ^{32}P	Time	Specific activity of corpuscle P (That of the inorganic plasma P taken to be 100)	
Intravenous injection throughout the experiment	215 min.	Inorganic P	12.7
		Average hydrolysable organic P	12.7
Subcutaneous injection throughout the experiment	11.5 hours	Inorganic P	25
		P obtained hydrolysis in 1 N H_2SO_4 at 100° for 15 hours	25
		P obtained by hydrolysis in 1 N H_2SO_4 at 100° for 120 hours	25
		Acid soluble residual P	13
	9 days	Total acid soluble P ...	94
	50 days	Total acid soluble P ...	100

TABLE 2. — DISTRIBUTION OF ^{32}P BETWEEN PLASMA AND CORPUSCLES OF THE HEN IN EXPERIMENTS IN VITRO AT 37°

	Time in hours	Percentage ^{32}P found in the corpuscles	Distribution coefficient of ^{32}P between corpuscle and plasma of equal weight
a)	1	6.05	0.14
	3	10.8	0.26
b)	2.3	4.2	0.15
	2.2(1)	3.2	0.11
c)	1.7	5.0	0.12
	4.7	6.3	0.23

(1) Corpuscles washed with NaCl solution.

PENETRATION OF PHOSPHATE INTO NUCLEATED CORPUSCLES

The rate of interchange between plasma phosphate and corpuscle phosphate was found to be much slower in hen's blood than in blood containing non-nucleated erythrocytes. The rate of renewal of the organic P compounds was also found to be slower in the corpuscles of the hen. The distribution of ^{32}P between plasma and corpuscles of the hen is seen in Table 2.

Table 3. — Extent of renewal of Acid soluble P Compounds in the Corpuscles of the Hen in the Course of 4.7 Hours at 37°

Fraction	P content mgm per cent	Relative specific activity	
Plasma inorganic P	6.67	100	
Corpuscle inorganic P ...	4.71	6.25	100
Hydrolysed during 7 min. .	2.68	4.48	72
Hydrolysed 7—180 min. .	25.1	2.06	33
Non-hydrolysed	31.6	0.77	12.3

The P atoms present in the nucleated erythrocytes of the frog were found to be replaced at a very slow rate, as seen in Table 4.

Table 4. — Distribution of ^{32}P between Plasma and Corpuscles of the Frog in Experiments in vivo

Time in hours	Temperature	Distribution coefficient of ^{32}P between corpuscles and plasma of equal weight
10	15°	0.28
14	20°	1.10
50	20°	1.40

RATE OF PENETRATION OF ^{32}P INTO THE CORPUSCLES IN THE PRESENCE OF EXCESS PLASMA PHOSPHATE

In two experiments, we added besides ^{32}P an appreciable amount of additional sodium phosphate to the blood increasing the inorganic phosphate content of the plasma to several times its normal value. As seen in Table 5, the rate of penetration of ^{32}P into the corpuscles is not much influenced by a very substantial increase in the phosphate concentration of the plasma. This result is to be interpreted in the follow-

ing way. The number of phosphate ions penetrating into the corpuscles is proportional to the number of phosphate ions present in the plasma. If no excess phosphate is added to the plasma for each phosphate ion penetrating into the corpuscles a phosphate ion will move from the corpuscles into the plasma, since otherwise a constant decrease in the phosphate concentration of the plasma would take place. That an incessant interchange of phosphate between plasma and corpuscles takes place can be shown by adding labelled phosphate of negligible weight to the plasma. As long as the ^{32}P concentration of the corpuscle inorganic P is lower than the ^{32}P concentration of the plasma inorganic P — what is always the case, except for experiments of long duration — a ^{32}P atom penetrating from the plasma into the corpuscles will be replaced by a ^{31}P atom moving in the opposite direction and, correspondingly, an accumulation of ^{32}P will take place in the corpuscles. If, by replacing a portion of the plasma chloride by phosphate, the phosphate concentration is raised to twice its original value, during the same time twice as many P atoms will penetrate from this plasma into the corpuscles than from the normal plasma. The radio-phosphate ions moving from the plasma into the corpuscles will now be replaced partly by stable phosphate and partly by chloride or other anions present in the corpuscles. Since the stable phosphate obviously behaves in the same way as the radiophosphate, the phosphate ions penetrating into the corpuscles will be replaced by phosphate, chloride or other anions previously located in the corpuscles. The amount of phosphorus replaced in the plasma by chloride (other anions) originally present in the corpuscles can be determined by measuring the decrease in the P content of the plasma, the amount of plasma P replaced by corpuscle P, however, by determining the percentage of ^{32}P which is to be found in the corpuscles. If we find, for example, by radioactive measurement that 20 per cent of the ^{32}P and by chemical determination that 10 per cent of the ^{31}P left the plasma after raising the plasma phosphate concentration by adding some excess phosphate and ^{32}P, we can conclude that half of the

Table 5. — Distribution of ^{32}P between Plasma and Corpuscles of the Rabbit in Experiments in vitro at 37°

	Time in min.	P added	Per cent of ^{32}P added found in the corpuscles
a)	70	^{32}P of negligible weight	19.7
	70	^{32}P + 8 fold increase of plasma phosphate	21.4
b)	90	^{32}P of negligible weight	32.0
	90	^{32}P + 7 fold increase of plasma phosphate	32.1

phosphate ions which penetrated into the corpuscles were replaced by phosphate ions located previously in the corpuscles (interchange) while the other half was replaced by other anions than phosphate located previously in the corpuscles (accumulation).

In the first experiment, by adding active phosphate buffer to the blood, we increased the inorganic P content of the plasma from 3.8 to 30.8 mgm per cent. In the second experiment, 0.1 cc. isotonic active phosphate buffer was added to 3 cc. blood, increasing thus the phosphate content of the plasma to 7.3 times its normal value. In the test experiment, 3 cc. blood was shaken with 0.1 cc. isotonic NaCl solution containing ^{32}P of negligible weight.

From 100 ^{32}P atoms added, the corpuscles contained 19.7 and 21.4 atoms, respectively, in the first experiment, and 32.0 and 32.1 atoms, respectively, in the second experiment. These figures state obviously the percentage of phosphate ions which migrated in the course of the experiment from the plasma into the corpuscles, but they supply no information about the reversed process and, thus, no information on the problem how much excess phosphate was accumulated in the corpuscles. This question is discussed on p. 507.

EFFECT OF TEMPERATURE ON THE RATE OF PENETRATION OF PHOSPHATE INTO THE CORPUSCLES

Early workers (Ege, 1919, Iversen, 1921) arrived at the result that the corpuscle membrane of human and rabbit blood is only slightly permeable to phosphate at 3° and that this permeability increases somewhat with a rise in temperature. Halpern (1936) found later that phosphate diffuses across the corpuscle membrane not at all at 3° and only at a very slow rate at 23°; at 37.5°, however, the rate of diffusion is greatly accelerated. Penetration of phosphate into the corpuscles was tested by noting whether inorganic phosphate added to the blood would enter the corpuscles or whether it would be carried out of the corpuscles with water after the addition of hypertonic sodium chloride or sucrose solutions to the blood.

The application of radiophosphorus in permeability studies leads to the result that the amount of phosphate penetrating the corpuscle membrane is much smaller at 0° than at 37°, the permeability being, however, easily determinable even at 0°. The results of such studies are seen in Table 6.

In the experiments lasting 64 and 72 minutes, in contrast to our usual procedure, we washed the corpuscles once with 0.9 per cent sodium chloride solution. As mentioned on p. 493, the centrifuged corpuscles contain some plasma and some of the ^{32}P found in the corpuscle fraction

is present in the adhering plasma. While, usually, this ^{32}P can be disregarded, that is not the case in experiments of short duration carried out at low temperatures. It is, therefore, of importance to remove the adhering plasma in such experiments. In the experiment lasting 7 hours, we omitted washing of the corpuscles. The same amount of ^{32}P which penetrated into the corpuscles in the course of 7 hours at 0° was found to be present at 37° after about $\frac{1}{2}$ hour. By increasing the temperature from 0° to 37° the rate of penetration of phosphate into the corpuscles thus increases about 14 times.

Table 6. — Effect of Temperature on the Rate of Penetration of ^{32}P into the Corpuscles of the Rabbit

Time	Temperature	Per cent of ^{32}P added to the blood present in the corpuscles
19 min.	37°	12.2
31 „	37°	16.6
64 „	0°	1.29
66 „	37°	25.9
72 „	0°	1.24
72 „	37°	19.3
90 „	5°	3.26
90 „	37°	19.7
90 „	37°	25.7
97 „	37°	23.8
105 „	37°	22
7 hours	0°	14.6
7 „	0°	12.7

A marked effect of increasing temperature on the rate of penetration of phosphate can be expected in view of the fairly slow rate of penetration of phosphate into the corpuscles. Usually a low rate of diffusion or of a similar process goes hand in hand with the high temperature coefficient. The diffusion rate equals

$$D = A.S.e^{-\frac{Q}{RT}}$$

In this equation, A denotes the number of collisions, S the probability of energetically satisfactory encounters, Q the energy of activation, R the gas constant, and T the absolute temperature.

Since S is often = 1, the magnitude of $e^{-\frac{Q}{RT}}$ determines mainly the rate of diffusion. In the case of a slow penetration, $e^{-\frac{Q}{RT}}$ will be small and Q correspondingly large. The effect of a change of T on the value

of $e^{-\frac{Q}{RT}}$ and thus on the temperature coefficient will be very much larger if Q has a larger value than in the opposite case.

For example, if $\frac{Q}{RT} = 10$, the replacement of T by T/2 will result in a reduction of the value of $e^{-\frac{Q}{RT}}$ from $4.5 \cdot 10^{-5}$ to $2.1 \cdot 10^{-9}$, thus to less than 1/5000 of the initial value while, if $\frac{Q}{RT}$ is taken to be $= 1$, a corresponding change in T will result in a decrease of $e^{-\frac{Q}{RT}}$ from 0.37 to 0.14, only.

Assuming S = 1, the heat of activation of the penetration of phosphate (Q) can be calculated from the equation

$$\frac{D_{37^\circ}}{D_{0^\circ}} = \frac{e^{-\frac{Q}{R\,310}}}{e^{-\frac{Q}{R\,273}}}$$

and as $\frac{D_{37^\circ}}{D_{0^\circ}}$ was found to be = 14, Q works out to be 15000 cal.

INCORPORATION OF ^{32}P INTO ORGANIC COMPOUNDS IN BLOOD HEMOLYSATE

As we stated above (p. 496), the rate of incorporation of ^{32}P into some of the P compounds of the corpuscles is faster than the rate of penetration of ^{32}P through the corpuscle membrane. It is, thus, the rate of the last mentioned slower process which determines the rate of interchange of corpuscle P and plasma P as long as the specific activity of the corpuscle inorganic P remains behind the specific activity of the plasma inorganic P. This statement is based on the fact that, while the specific activity of the corpuscle pyrophosphate P, for example, some time after the start of the experiment not much differs from the specific activity of the corpuscle inorganic P, 1 mgm of corpuscle inorganic P is found to be much less active than 1 mgm of plasma inorganic P. This conclusion involves the assumption that the fraction secured from the de-proteinated corpuscles as inorganic phosphate was actually present as such in the corpuscles and not as a constituent of a not yet known labile P compound. This assumption may probably be correct. The distribution coefficient of free phosphate between plasma and corpuscles can be expected not to differ much from the distribution coefficient of chloride and other free anions between plasma and corpuscles. Thus, 1 gm of corpuscle can to be expected to contain somewhat less than half

as much free P as 1 gm of plasma[1]. This conclusion is born out by the experiment.

The process of incorporation of ^{32}P into organic phosphorus compounds can be studied in blood hemolysate free from permeability considerations. We hemolysed rabbit corpuscles by repeatedly cooling rabbit blood to liquid air temperature. The hemolysate was shaken for 2 hours at 37° with labelled phosphate of negligible weight. From the trichloroacetic filtrate, the inorganic P present as such in the hemolysate was precipitated as magnesium salt, the filtrate was then made 1 N H_2SO_4 and kept at 100° for 7 minutes. By this procedure, the labile P of the adenosintriphosphoric acid is split off. By a similar procedure, other P fractions were also secured. The results of these experiments are seen in Table 7.

TABLE 7. — SPECIFIC ACTIVITY OF THE P FRACTIONS OF RABBIT BLOOD HEMOLYSATE KEPT FOR 2 HOURS AT 37°

Fraction	P content in mgm	Percentage distribution of ^{32}P	Specific activity
Inorganic P	4.45	85	100
Hydrolysed 0—7 min.	2.06	9.2	23.5
Hydrolysed 7—100 min.	3.79	—	5.8
Hydrolysed 7 min.—17 hours	10.88	5.37	2.58
Residual acid soluble P	1.62	0.33	1.07
Total organic acid soluble P	14.6	14.9	6.29

In an other experiment the pyrophosphate fraction had a higher specific activity (41).

All acid soluble organic P fractions secured from the hemolysate were found to contain ^{32}P, *phosphorylation is thus occurring in the blood hemolysate*. The percentage of adenosintriphosphate, which is resynthesized in the hemolysate, is, however, smaller than the percentage resynthesized in the corpuscles during the same time. In the experiment with intact corpuscles, in the course of 1½ hours, the pyrophosphate P present in the corpuscles had nearly the same activity as the inorganic P present in the corpuscles. In the hemolysate, in the course of 2 hours, the activity of the pyrophosphate P amounted to only about ¼ to ½ of the activity of the inorganic P. In fact, the difference between the result obtained in the corpuscles and the hemolysate is even larger than stated above.

(1) We can expect

$$\frac{\overline{Cl}_{\text{corp. } H_2O}}{\overline{Cl}_{\text{pl. } H_2O}} = \frac{(a \times \overline{H_2PO_4^-})_{\text{corp. } H_2O} + (b \sqrt{\overline{HPO_4^{--}}})_{\text{corp. } H_2O}}{(a \times \overline{HP_2O_4^-})_{\text{pl. } H_2O} + (b \sqrt{\overline{HPO_4^{--}}})_{\text{pl } H_2O}}$$

where a denotes the fraction of phosphate being present as primary ion and b the fraction of phosphate being present as secondary ion.

In the hemolysate, in the course of the experiment the amount of inorganic P increases due to the successive decomposition of organic P compounds. This fact leads to a corresponding decrease of the specific activity of the inorganic P. When comparing the specific activity of the pyrophosphate P split off by hydrolysis with the specific activity of the inorganic P present in the hemolysate at the end of the experiment, we, therefore, overestimate the extent of renewal of the adenosintriphosphate.

The difference between the rate of resynthesis of the other P compounds in the hemolysate and in the corpuscles is still larger than that found in the case of adenosintriphosphate. In the corpuscles, after the lapse of $1\frac{1}{2}$ hours, the activity of the P obtained by hydrolysis for 12 hours after removal of the pyrophosphate amounted to 57 per cent of the activity of the inorganic P of the corpuscles. In the hemolysate, in the course of 2 hours, the corresponding figure was only 5.8 per cent. While, therefore, phosphorylation processes are going on in the hemolysate, their rate remains much behind the corresponding processes going on in the intact corpuscles.

That the rate of resynthesis of organic P compounds is strongly reduced in the hemolysate follows also from the fact that the hemolysate contains more inorganic P and less organic P than intact blood[1]. As shown by using labelled P as an indicator, an alternative degradation and resynthesis of adenosintriphosphate and also of other organic P compounds takes place in the corpuscles and also in the hemolysate. In the hemolysate the resynthesis obviously lacks behind the degradation and the inorganic P content correspondingly increases in the course of the experiment.

The rate of resynthesis of organic P compounds in the hemolysate is much reduced by lowering the temperature. As seen in Table 8, at 0°

Table 8. — Effect of Temperature on the Formation of Labelled Organic P Compounds in Rabbit Blood Hemolysate

Time in hours	Temp.	Percentage of ^{32}P added found in the organic fraction	P content in mgm p. c.		Specific activity	
			Inorganic P	Organic P	Inorganic P	Organic P
1	37°	11.6	9.38	15.0	100	8.20
	0°	3.34	9.05	15.3	100	2.00
2	37°	17.2	5.55	18.9	100	5.09
	0°	7.10	3.76	20.6	100	1.20

[1] A detailed study of the effect of hemolysis on the content of different P compounds present in horse blood was recently made by Sjöberg (1940), comp. also Solomon and co-workers (1940).

about $\frac{1}{4}$ as many labelled organic P compounds were synthesized as at 37°.

The lowering of the rate of formation of labelled adenosintriphosphate in the hemolysate is possibly due partly or wholly to destruction of cozymase taking place in the hemolysate. In a hemolysate of the corpuscles of horse blood LENNERSTRAND (1941) found, 3 hours after hemolysis, a destruction of 40 per cent of the cozymase present in the corpuscles. LENNERSTRAND found also that the increase of the inorganic phosphate content of the hemolysate is due at least partly to dephosphorylation of adenosintriphosphate. The composition of a mixture of hemolysate, cozymase and adenosintriphosphate was found, however, not to change.

RUNNSTRÖM, LENNERSTRAND *et al.* (LENNERSTRAND, 1941) observed in a hemolysate of corpuscles of horse blood a synthesis of organic phosphate occurring at the expense of the oxydation of hexosediphosphate. The reaction was found to require the presence of diphosphodinucleotide, coenzyme and methylenblue or another dye which acts as oxygen carrier. While dismutation and phosphorylation were found to take place in the hemolysate, all processes consecutive to the formation of monophosphoglyceric acid were found to be absent.

The intermediary formation of inorganic phosphate is a normal step in the phosphorylation circuit going on in the corpuscles. In the intact corpuscle the inorganic phosphate split off is soon incorporated into another organic molecule and its splitting off is thus hidden from observation, except from using an isotopic indicator which reveals that we are faced with a dynamic equilibrium. If the phosphorylation mechanism is disturbed in the corpuscle or in the hemolysate, incorporation of phosphate into organic compounds takes place at a reduced rate only and an accumulation of free phosphate takes place in the blood.

EFFECT OF POISONS ON THE ENZYMES RESPONSIBLE FOR THE SYNTHESIS OF ORGANIC P COMPOUNDS IN THE CORPUSCLES

In normal corpuscles, the penetration of ^{32}P into the corpuscle is followed by an incorporation into organic molecules which, in turn, give off nonlabelled P. By this process, the corpuscle inorganic P is prevented from obtaining for quite a while a high specific activity. If, however, a formation of organic P compounds is hindered, and this should be the case in a poisoned corpuscle, the ^{32}P migrating into the corpuscle would remain in the inorganic P fraction and this fraction would soon become strongly active.

We failed to inhibit the process of resynthesis by adding fluoride to rabbit blood, as seen in Table 9. 2 cc. blood + 0.5 cc. 0.2 mol sodium

TABLE 9. — DISTRIBUTION OF ^{32}P BETWEEN PLASMA AND CORPUSCLES AFTER 159 MIN IN THE ABSENCE AND PRESENCE OF FLUORIDE

	Percentage distribution of ^{32}P	
	Plasma	Corpuscles
Control	57.2	42.8
Fluoride	56.4	43.6

fluoride solution were first shaken at 37° for 87minutes. ^{32}P was then added and the blood was kept for further 159 minutes at 37°. In the control experiments, the sodium fluoride solution was replaced by RINGER's solution. Investigating phosphorylation in the hemolysate of the corpuscle of the horse, LENNERSTRAND (1940) found that addition of fluoride to the hemolysate does not inhibit phosphorylation, though the rate of phosphorylation gets somewhat reduced.

The addition of KCN to the blood, however, had a marked though highly varying effect on the system of resynthesis. Rabbit blood was shaken with different volumes (see Table 10) of an isotonic potassium cyanide solution (pH = 7.5—8.0) at 37° for some time previous to addition of labelled phosphate of negligible weight. As seen in Table 10, the corpuscles treated in this way took up less labelled phosphate than the corpuscles which were not treated with cyanide. Furthermore, the distribution of ^{32}P between the different phosphorus compounds present in the corpuscles was different in the controls and in the cyanide treated erythrocytes. In the corpuscles treated with cyanide, most of the ^{32}P present was in the inorganic fractions. From this result follows that it is not so much the permeability of the corpuscles which is influenced by the presence of cyanide as the phosphorylation process occurring in the corpuscles.

TABLE 10. — UPTAKE OF ^{32}P BY THE CORPUSCLES OF THE RABBIT AT 37° AFTER TREATMENT WITH CYANIDE SOLUTION AT 37°

Mgm KCN added per cc. of blood	Shaken previous to the addition of labelled phosphate min.	Shaken after addition of labelled phosphate min.	Percentage uptake of ^{32}P by the corpuscles	Percentage distribution of ^{32}P between the P fractions of the corpuscles	
				Inorganic P	Organic P
—	—	97	23.8	30	70
—	—	101	25.5	34	66
—	—	105	22.0	19	81
0.1	78	95	17.5	52	48
1.5	22	64	16.8	—	—
1.5	92	101	13.5	91	9
2.0	155	102	15.6	60	40
2.0	51	97	12.8	56	44

ACCUMULATION OF PHOSPHATE IN THE CORPUSCLES

As already mentioned, the *interchange* of P atoms between plasma and corpuscles is not restricted to the P atoms of the free phosphate present in the corpuscles, in contrast to the *accumulation* of additional phosphate. If we increase the phosphate concentration of the plasma we may expect to obtain, after a sufficiently long time, a corresponding increase in the free phosphate concentration of the corpuscles. The exact determination of the free phosphate content of the corpuscles encounters some difficulties, since even a slight decomposition of the large amounts of organic phosphorus compounds present in the corpuscles during the separation and extraction processes will appreciably influence the amount of free phosphate found to be present in the corpuscles. This fact may be partly or wholly the reason why such varying values are found for the inorganic phosphate concentration of the corpuscles[1]. The average value found for the free P content of the corpuscles amounts to about 1.5 mgm per cent. Since rabbit blood is composed of about 2 parts of plasma and 1 part of corpuscles and the corpuscles contain somewhat less than $\frac{1}{2}$ as much inorganic P as 1 gm plasma, we can expect from the phosphate added to the blood about $^1/_5$ to penetrate into the corpuscles.

We determined the increase in the phosphate concentration of the corpuscles due to addition of phosphate to the plasma in the following experiments. To 2 cc. samples of rabbit blood 0.04 cc. phosphate buffer of physiological concentration was added. Half of the samples obtained was centrifuged at once, while the other half was first shaken for $2\frac{1}{2}$ hours in a O_2—CO_2 atmosphere at 37°. Both plasma samples obtained were precipitated with 5 per cent trichloroacetic acid and the P content of the filtrate obtained was determined by the method of Fiske and Subbarow. The result obtained is seen in Table 11.

Thus, 1.54 mgm per cent or 12.5 per cent of the plasma phosphate, corresponding to 17.6 per cent of the excess phosphate added, left the plasma for the corpuscles in the course of $2\frac{1}{2}$ hours. During the same time, 35 per cent of the ^{32}P added to the plasma was found by radioactive measurement to have penetrated into the corpuscles.

As mentioned on p. 498, this difference may be interpreted in the following way. 12.5 per cent of the phosphorus atoms present at the start of the experiment in the plasma accumulated during the experiment in the corpuscles, increasing the inorganic P content of the erythrocytes. Besides this accumulation, a replacement of a part of the inorganic P and of the organic P of the corpuscles by plasma P (interchange) took place as well. The percentage P atoms present at the start of the experiment in the plasma, which reached the corpuscles at the end of the expe-

[1] Comp. for example, L. Halpern (1936).

TABLE 11. — DISTRIBUTION OF PHOSPHATE ADDED TO THE BLOOD BETWEEN PLASMA AND CORPUSCLES

Sample	Acid soluble P content of the plasma in mgm. per cent	
	At the start	2½ hours later
I	12.20	10.66
II	12.12	10.72
III	12.67	10.74
IV	12.30	10.73
V	12.16	10.70
VI	12.21	10.73
VII	12.25	10.80
VIII	12.28	10.73
IX	12.27	10.73
Average value	12.27	10.73

riment, is given by the percentage ^{32}P found at the end of the experiment in the corpuscles. By subtracting from this figure (35 per cent) the percentage of plasma P which accumulated in the corpuscles we arrive at the percentage of plasma P which reached the corpuscles solely by interchange (replacement). This percentage is found to be 22.5.

In another experiment, to 17 cc. of rabbit blood 0.3 cc. phosphate buffer containing 3.8 mgm of P per cc. was added. An aliquot of the blood was centrifuged at once, another one was shaken at 37° for 2½ hours. The determination of the plasma phosphate gave the following results.

TABLE 12. — DISTRIBUTION OF PHOSPHATE ADDED TO THE BLOOD BETWEEN PLASMA AND CORPUSCLES OF THE RABBIT

Acid soluble P content of the plasma in mgm per cent		
At the start		2½ hours later
14.4, 14.4, 14.7	I	11.7
	II	12.0
	III	11.8
	IV	12.6
Average 14.5		12.0

Thus, after increasing the plasma P content from 3.5 mgm per cent to 14.5 mgm per cent, 2.5 mgm per cent P corresponding to 23 per cent of the phosphate added left the plasma and accumulated in the corpuscles.

Since the total acid soluble P content of the corpuscles is about 80 mgm per cent, an increase in the P content of the corpuscles amounting to 5 mgm increases the total P content of the erythrocytes with 6 per cent, only[1].

In contrast to the excess phosphate added, the plasma ^{32}P gets distributed between most or all of the acid soluble P of the plasma and the total acid soluble P of the corpuscles. The organic molecules undergo enzymatic degradation and resynthesis and, in the course of this process, a replacement of organic P by inorganic P and, thus, by labelled P takes place. An increase in the amount of organic P compounds necessitates besides the presence of P excess an excess of all other ingredients necessary to the formation of such compounds. These are, however, available to a small extent only in the corpuscles. This fact makes it easily understandable that the whole plasma P ultimately interchanges with corpuscle P, while an increase in the concentration of plasma P leads but to a minor increase in the concentration of the total corpuscle P.

RATE OF PHOSPHATE ACCUMULATION IN THE CORPUSCLES

From the fact that, after the lapse of a few hours, the specific activity of the free P of the corpuscles does not differ to any appreciable extent from the specific activity of most of the acid soluble organic P fractions present in the corpuscles, while the specific activity of the free plasma P is found to be much higher than the specific activity of the corpuscle P, we have drawn the conclusion that the penetration of phosphate through the corpuscle membrane is a fairly slow process. This result was checked by measuring the rate of intrusion of excess phosphate added to blood into the corpuscles. The phosphate concentration of rabbit plasma was raised by addition of phosphate buffer from 3.7 to 16.5 mgm per cent

Table 13. — Rate of Penetration of Excess Phosphate added to Blood into the Corpuscles

Time in min	Inorganic P content of plasma in mgm. per cent
0	16.5
13.5	16.3
52.5	15.6
141	14.4

[1] In carrying out the above calculations, the blood is assumed to be made up of two parts of plasma and one part of corpuscles.

and the decrease of the phosphate content of the plasma was determined at different intervals. As seen in Table 13, the excess phosphate penetrated at a fairly slow rate, only, into the corpuscles.

In certain experiments[1], inorganic P was found to leave the corpuscles and to enter the plasma even though the concentration in the plasma exceeded that in the corpuscles. This fact was interpreted as an indication that the transfer of substances across the corpuscle membrane cannot always be explained by the simple physico-chemical law of diffusion from a higher to a lower concentration, even with the modification described by DONNAN. The correctness of this interpretation can be challenged. The concentrations of inorganic phosphate in the corpuscle water and the plasma water differ for the same reason as the concentrations of the chloride ions in the erythrocytes and in the plasma differ. In equilibrium, 1 gm of plasma contains about twice as much chloride, respectively phosphate, as 1 gm of corpuscles. If, in the course of phosphorylation processes going on in the corpuscles, inorganic phosphate is made free, the equilibrium between corpuscle phosphate and plasma phosphate is upset and a part of the additional phosphate migrates into the plasma in spite of the fact that the phosphate concentration in the plasma is higher than in the corpuscles. The migration will come to an end as soon as the equilibrium ratio between plasma phosphate and corpuscle phosphate is obtained, a ratio which is somewhat less than 2, as mentioned above. The phosphate content of the plasma constantly changes, it increases if the phosphate taken up with the food gets absorbed, when bone apatite gets dissolved, when organic P compounds present in the tissue cells or corpuscles get decomposed, it decreases when glucose is absorbed into the circulation, when additional ossification takes place, when inorganic phosphate present in the tissue cells or in the corpuscles gets incorporated into organic molecules, and so on. An increase in the phosphate concentration of the plasma will lead to an influx of some phosphate into the corpuscles and a decrease to a movement in opposite direction. In contrast to the behaviour of chloride which responds almost momentarily to changes in the chloride concentration of the plasma (510), phosphate does not. The rate of passage of phosphate through the corpuscle membrane is fairly slow, it will take an appreciable time before equilibrium between the concentration of the plasma phosphate and the corpuscle phosphate is obtained. In the meantime, changes in the concentration of phosphate in the plasma will often take place which are independent of the happenings going on in the corpuscles. For these reasons, the ratio of plasma phosphate and corpuscle phosphate may never reach an equilibrium state but, nevertheless, the transfer of phosphate across the cell membrane can well be explained

[1] Comp. L. HALPERN (1936).

by the simple physico-chemical law of penetration. In such consideration we must always envisage the fact that it is not the concentration difference of the ion in the corpuscle water and the plasma water which matters, but the deviation from the equilibrium distribution ratio which can, and often does, deviate considerably from unity.

A closer analysis of the happenings in the corpuscles reveals the following facts. The high phosphate content of the corpuscles is due to an incorporation of a very substantial amount of phosphate in the molecules of adenosintriphosphate, hexosemonophosphate, diphosphoglycerate, and so on. These molecules are incessantly degraded and rebuilt in the course of the metabolic circle going on in the corpuscles. The larger part of the phosphate present in the corpuscles is thus incorporated into organic molecules and is no longer free phosphate. The maintenance of the great difference in the acid soluble P content of the corpuscles and of the plasma is thus dependent on the metabolic circle going on in the corpuscles. The accumulation of ions in plant and animal cells is found to be generally conditioned by metabolic processes going on in the organism; in the case of concentration of phosphate in the corpuscles the way in which the metabolic process performs the ionic concentration is clearly shown, while we are in the dark in other cases as to the mechanism of the process of accumulation. *This mechanism may be built up in some other cases on similar lines as described above.*

RATE OF PENETRATION OF LABELLED CHLORIDE INTO THE CORPUSCLES

We arrived at the result that phosphate penetrates at a fairly slow rate into the corpuscles. When making this statement we compare the rate of penetration of phosphate with the rate of intrusion of chloride. Chloride ions are known to penetrate speedily into the corpuscles. The increase of the CO_2 content of the blood is followed by an increase in the bicarbonate content of the erythrocytes. As a result of the increase in the bicarbonate content of the corpuscles, bicarbonate will move from the erythrocytes into the plasma and chloride from the plasma into the corpuscles. The interchange between chloride and bicarbonate which is intimately connected with respiration processes is very fast, in about 2 seconds half of the equilibrium distribution is reached (Dirken and Mook, 1931).

In view of the very small resistance the chloride ions encounter when penetrating the corpuscle membrane, we can expect the chloride ions not only to interchange speedily with HCO_3^- ions but also with other Cl^- ions. Labelled Cl^- ions introduced into the plasma will soon be found in the corpuscles, and vice versa.

Mr. ZERAHN has investigated in our laboratory the rate of penetration of radiochloride into the corpuscles of the rabbit. The radiochloride was prepared by bombarding carbontetrachloride with neutrons and by extracting the radiochloride produced by the method of SZILARD and CHALMERS. A small volume of a physiological sodium chloride solution prepared from the chloride obtained by the process described above was added to rabbit blood. Since the rate of penetration was expected to be very rapid the experiments were carried out at 0° and the mixture was shaken for 1 min only, previous to centrifuging.

To the active corpuscles separated by centrifuging the corresponding amount of non-active plasma was added, while the active plasma was mixed with a corresponding amount of non-active corpuscles. Two blood samples of the same weight were thus obtained, one of which contained active corpuscles and the other active plasma. The blood samples obtained by this procedure (about 100 mgm) were mixed with 100 mgm of CaO powder and were placed under the Geiger counter. Since the ^{39}Cl used as an indicator has a half-lifetime of only 38 min, this procedure has obvious advantages over the usual process in which the blood samples are ashed or extracted. In these as in all other experiments, the sharply centrifuged corpuscles were not washed, since washing might lead to a substantial loss of labelled ions by the corpuscles.

1 gm of corpuscle was found to contain about half as much ^{39}Cl as 1 gm of plasma. Since the chloride content of 1 gm of corpuscle of the rabbit makes out 0.53 times the chloride content of 1 gm of plasma (ADLERSBERG and GLASS, 1932), in the course of 1—3 min.[1] even at 0° a very large portion of the corpuscle chloride was replaced by labelled chloride originally present in the plasma. The rate of penetration of chloride into the corpuscles of the rabbit is, thus, more than 100 times larger than the rate of penetration of phosphate.

PENETRATION OF LABELLED SODIUM INTO THE CORPUSCLES OF THE RABBIT

In our first experiments (HAHN, HEVESY and REBBE, 1939), labelled sodium was administered to rabbits as sodium chloride by subcutaneous injections. After the lapse of 1 day, we found 1 gm of sharply centrifuged, unwashed corpuscles to have a ^{24}Na content amounting to 14 per cent of that of 1 gm of plasma. From these 14 per cent a few per cent are due to the ^{24}Na content of the adhering plasma while the rest is due to ^{24}Na penetrated into the corpuscles. The distribution ratio of ^{24}Na

[1] As the separation of the corpuscles from the plasma by centrifuging lasts some time, the duration of the experiment was somewhat more than 1 min.

between plasma and corpuscles did, thus, not much differ from the corresponding distribution ratio of ^{23}Na as the sodium content of 1 gm of corpuscle amounts to about $\frac{1}{9}$ of the sodium content of 1 gm of plasma. The specific activity of the sodium of the corpuscle did, thus, not much differ from the specific activity of the sodium of the plasma. Hence, we have to conclude that in the course of the experiment the major part or all of the corpuscle sodium interchanged with plasma sodium.

Experiments of short duration in which labelled sodium was administered by intravenous injection to the rabbit showed that the rate of interaction of the plasma sodium with the corpuscle sodium is not slow. After the lapse of 15 minutes, the activity of 1 gm of corpuscles was found to amount to 11 per cent of that of 1 gm of plasma (Table 14).

In another experiment, after the lapse of 11 minutes, the percentage ratio was found to be 9.

The comparison of the permeability of the wall of the corpuscles of the rabbit to sodium and to chloride is made difficult by the fact that the sodium content of the rabbit corpuscles is very low and amounts to only about $\frac{1}{9}$ of the sodium content of the plasma. The amount of labelled sodium adsorbed on the corpuscle membrane may, therefore, constitute quite an appreciable part of the labelled sodium found in the

Table 14. — Distribution of ^{24}Na administered by Intravenous Injection between Corpuscles and Plasma of the Rabbit

Time in min.	Percentage distribution ratio of ^{24}Na between corpuscles and plasma of equal weight
2.3	5.7
15.1	11
32	10
61	10
120	11

centrifuged corpuscles. Furthermore, when only a fraction of the sodium ions of the plasma have penetrated into the corpuscles a proportional distribution of ^{24}Na between plasma and corpuscles will be reached, which is not the case for ^{39}Cl, since the concentration of chloride in the plasma and the corpuscle water differs only to a minor extent. The corpuscle water of the dog contains, however, nearly as much sodium as the plasma water and, therefore, the corpuscles of the dog and also the corpuscles of the cat are more suitable to be used in experiments in which the permeation of sodium is to be compared with the permation of chloride. W. E. and E. T. Cohn (1939), who where the first to determine

the permeability of the corpuscles of the dog to sodium, found that, in the course of a few hours, an appreciable part of the corpuscle sodium is replaced by plasma sodium. Similar values to those found by W. E. and E. T. Cohn (1939) for the penetration of sodium into the corpuscles

Table 15. — Distribution of ^{24}Na between Corpuscles and Plasma of Dog Blood Shaken With Labelled Sodium Chloride at 37°

	Time in min	Percentage of ^{24}Na added present in the corpuscles	Percentage distribution ratio of ^{24}Na between corpuscles and plasma of equal weight
Dog A	5.1	7.9	9.6
	15.6	12.0	14.6
	57.0	15.9	19.4
Dog B	4.0	4.9	6.6
	8.3	6.6	9.0
	13.6	8.0	10.9
	27.9	8.4	11.4
	41.2	11.7	15.8
	65.5	11.6	15.7
Dog C	10.5	8.2	10.0
	31	11.0	13.4
	85	14.9	18.1

Table 16. — Distribution of ^{24}Na Administered by Subcutaneous Injection Between Corpuscles and Plasma of the Cat

	Time in hours	Percentage distribution ratio of ^{24}Na between corpuscles and plasma of equal weight
Cat A	0.5	9.8
	1.1	10.4
	2.1	19.2
Cat B	0.5	10.2
	1.5	18.6
	3.0	28.4
	4.5	32.9
	6.0	36.4

of the dog were obtained in our experiments, the results of which are given in Table 15 and Fig. 1. The rate of penetration of sodium into the corpuscles is lower than the rate of penetration of phosphate. While after the lapse of 1 hour, thus, in experiments of comparatively short duration at 37° the distribution ratio of ^{32}P between corpuscles and plasma is formed to be 0.6, the corresponding figure for ^{24}Na is about 0.18.

Summary

The red corpuscles contain much larger amounts of acid soluble phosphorus compounds than the plasma. The difference is only maintained as long as an active process, an alternative phosphorylation and dephosphorylation, takes place in the corpuscles, a process which was studied by measuring the rate of penetration of labelled phosphorus into the erythrocytes and the rate of incorporation of ^{32}P into the acid soluble P compounds present in the corpuscles.

While labelled phosphate penetrates at about the same rate into the corpuscles of the human and the rabbit, it enters at a much slower rate the nucleated corpuscles of the hen and the frog.

The amount of labelled phosphate which penetrates into the corpuscles of the rabbit at 0° was found to make out about $\frac{1}{14}$ of the amount found at 37°.

Incorporation of labelled phosphate into acid soluble P compounds, thus alternative dephosphorylation and phosphorylation, was found to go on in the blood hemolysate as well, though at a slower rate than in the intact corpuscles.

A lowering of the temperature from 37° to 0° reduces the amount of labelled P incorporated into organic P compounds of the hemolysate to $\frac{1}{4}$.

The addition of KCN to blood reduces the formation of organic P compounds in the corpuscle markedly. The rate of penetration of phosphate into the corpuscles was also measured in accumulation experiments in which a part of the plasma chloride was replaced by phosphate and the amount of phosphate which left the plasma determined at different intervals.

The difference between the interchange of labelled and non-labelled ions, present in the plasma and the corpuscles respectively, and the accumulation of ions in the corpuscles after raising the ionic concentration of the plasma is discussed and it is shown that only interchange experiments supply a direct measure of the hindrance of a phase boundary to the passage of ions.

By using ^{32}P and ^{38}Cl, respectively, as indicators the percentage of plasma phosphate which penetrates per unit time into the corpuscles of the rabbit was found to be at least 100 times smaller than the percentage of plasma chloride penetrating into the corpuscles. The rate of penetration of sodium into the corpuscles of the dog using ^{24}Na as a tracer, was found to be slower than the rate of intrusion of phosphate.

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Comment on papers 49 and 50

The first investigations (paper 49) on the intrusion rate of P^{32} into red corpuscles brought out that even after several hours of incubation of blood in the presence of labelled sodium phosphate the specific activity of the corpuscular inorganic P lags very much behind the specific activity of the plasma inorganic P. After a very short time, however, the specific activity of the labile acid-soluble P of the corpuscles does not differ much from that of their inorganic P. This was interpreted as indicating a fairly slow penetration of P^{32} into the corpuscles followed by a rapid interaction with the labile P of the acid-soluble P compounds present in these. In more recent years Gourley and also Gerlach *et al.* measured specific activity values of the labile P of ATP of the red corpuscles which were found to be larger than the specific activity of the inorganic P isolated from them These observations suggest the explanation that at least some of the plasma inorganic P is incorporated into corpuscle ATP prior to its having the opportunity to be "diluted" by the inactive inorganic P of the corpuscles, the incorporation thus taking place in or on the phase boundaries. The isolation of corpuscle P is a difficult task, as a part of the small amounts of isolated inorganic P from the corpuscles may be an artifact, due to a splitting of a minute fraction of the large amounts of organic P present in the corpuscles in the course of the extraction process. The above mentioned results must therefore be carefully interpreted. As mentioned on p. 365 the synthesis of ATP in or on the phase boundaries of liver cells was made very probable by Sacks.

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End Of
Volume One